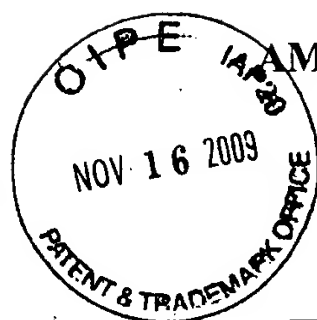


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AMENDED APPEAL BRIEF

Attorney Docket No. 31580-702.201

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

<p>In re the Application of:</p> <p>Applicants: Defu Zeng</p> <p>Serial No.: 09/844,544</p> <p>Filed: April 27, 2001</p> <p>Title: Methods for inhibition of polyclonal B cell activation and immunoglobulin class switching to pathogenic autoantibodies by blocking CD1-mediated interactions</p>	<p>Confirmation No.: 3043</p> <p>Group Art Unit: 1644</p> <p>Examiner: Marianne NMN Dibrino</p> <p>Customer No. 021971</p>
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MAIL STOP APPEAL BRIEF - PATENTS
Commissioner for Patents
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Alexandria, VA 22313-1450

AMENDED APPELLANTS' BRIEF PURSUANT TO 37 C.F.R. § 41.37

Appellants submit this amended brief in response to the Notification of Non-Compliance with 37 CFR 1.192(c) mailed on October 15, 2009. To comply with the requirements of the Notification, the Appeal Brief is herewith re-submitted without Figure 1 from Dr. Strober's Declaration.

This Amended Appeal Brief is submitted within one month from the mailing of the Notification; therefore, this Appeal Brief is timely filed.

I. REAL PARTY IN INTEREST

The real party in interest is The Board of Trustees of the Leland Stanford Junior University (Assignee) by virtue of an assignment executed by the inventors (Appellants) to The Board of Trustees of the Leland Stanford Junior University recorded by the Assignment Branch of the U.S. Patent and Trademark Office on April 27, 2001 at Reel 011771 and Frame 0601.

II. RELATED APPEALS AND INTERFERENCES

Appellants are unaware of any related appeals or interferences.

III. STATUS OF CLAIMS

The present application was filed on April 27, 2001 with original claims 1-14 pending. Following the Restriction Requirement of October 2, 2002, claims 3, 11 and 14 were withdrawn from consideration. In Applicants response of June 6, 2003, claims 4, 5 and 9 were cancelled. In Applicants response of December 19, 2003, claims 3, 11 and 14 were cancelled. In Applicants response of March 24, 2006, claim 2 was cancelled and claims 15-22 were added. In Applicants response of May 5, 2006, claims 1, 6-8, 10, 12 and 13 were cancelled. In Applicants response of October 20, 2006, claims 23-26 were added.

The rejections of claims 15-26 are being appealed.

IV. STATUS OF AMENDMENTS

Appellants have submitted no amendments after the non-final Office Action of February 6, 2008. All amendments prior to filing the Notice of Appeal were entered.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed subject matter in this appeal relates to novel methods to treat systemic lupus erythematosus in a human patient with a CD1d blocking antibody.

Claim 15

The subject matter claimed in independent claim 15 is a method of treating systemic lupus erythematosus in a human patient comprising administering an effective dose of a CD1d blocking antibody.

Support for a method of treating systemic lupus erythematosus in a human patient is found in the specification at least in paragraphs 0001-0007, 0051, and 0061; and claim 2 as originally filed.

Support for administering to said patient an effective dose of a CD1d blocking antibody is found at least in the specification in paragraphs 0031-0033, 0035, 0059, 0063, and 0065; and claim 1 as originally filed.

Support for said effective dose treating said systemic lupus erythematosus in said human patient is found at least in paragraphs 0087, 0088, and 0095.

Claim 23

The subject matter claimed in independent claim 23 is a method of treating systemic lupus erythematosus in a human patient comprising administering to said patient an effective dose of a CD1d blocking antibody and wherein the effective dose is sufficient to inhibit a pathological polyclonal B cell activation or class switching.

Support for a method of treating systemic lupus erythematosus in a human patient is found in the specification at least in paragraphs 0001-0007, 0051, and 0061; and claim 2 as originally filed.

Support for administering to said patient an effective dose of a CD1d blocking antibody is found at least in the specification in paragraphs 0031-0033, 0035, 0059, 0063, and 0065; and claim 1 as originally filed.

Support for said effective dose treating said systemic lupus erythematosus in said human patient is found at least in paragraphs 0087, 0088, and 0095.

Support for the inhibition of pathologic polyclonal B cell activation or class switching is found in at least the Title, Abstract, paragraphs 0001, 0007, 0012, 0018, 0022, and 0088; Figure 5; and claims 1 and 2 as originally filed.

Claim 24

The subject matter claimed in independent claim 24 recites a method of treating systemic lupus erythematosus in a human patient comprising administering to said patient an effective dose of a CD1d blocking antibody, wherein the effective dose is sufficient to reduce proteinuria.

Support for a method of treating systemic lupus erythematosus in a human patient is found in the specification at least in paragraphs 0001-0007, 0051, and 0061; and claim 2 as originally filed.

Support for administering to said patient an effective dose of a CD1d blocking antibody is found at least in the specification in paragraphs 0031-0033, 0035, 0059, 0063, and 0065; and claim 1 as originally filed.

Support for said effective dose treating said systemic lupus erythematosus in said human patient is found at least in paragraphs 0087, 0088, and 0095.

Support for delaying the onset of proteinuria is found at least in paragraphs 0012, 0018, 0067, and 0088; and in Figure 5.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Appellants respectfully request the Board of Patent Appeals and Interferences to review the following grounds of rejection on appeal:

1. Whether claim 22 complies with the written description requirement under 35 U.S.C. 112, first paragraph.
2. Whether claims 15-20 and 23-26 are patentable under 35 U.S.C. 103(a) over Amano et al. J. Immunol. 1998, 161: 1710-17 ("Amano"), in view of Kotzin, Cell, 1996; 85: 303-306 ("Kotzin"), Zeng et al., J. Exp. Med. 1998, 187:525-36 ("Zeng"), Blumberg et al., Immunol. Rev. 1995, 147:5-29 ("Blumberg) and Hughes, Drug Discov. Today, 1998; 3(10):439-42 ("Hughes").
3. Whether claims 21 and 22 are patentable under 35 U.S.C. 103(a) over Amano in view of Kotzin, Zeng, Blumberg, Hughes and Merck Manual, 1992, 16th Edition, pages 1317-21 ("Merck Manual").
4. Whether claims 15-26 are patentable under 35 U.S.C. 103(a) Amano in view of Kotzin, Zeng, U.S. Patent No. 6,531,453 (the '453 patent), Blumberg and Hughes.
5. Whether claims 15-26 are patentable under 35 U.S.C. 103(a) Amano in view of Kotzin, Zeng, the '453 patent, Blumberg, Hughes and the Merck Manual.

VII. APPELLANTS' ARGUMENTS

Appellants respectfully submit that the specification provides sufficient written description for claim 22. In addition, Applicants respectfully submit that claims 15-26 are in proper form and are patentable over the prior art of record.

1. Claim 22 Complies with the Written Description Requirement Under 35 U.S.C. § 112, First Paragraph with Respect to an Immunomodulating Drug

The Examiner has rejected claim 22 as lacking sufficient written description. Claim 22 is directed to a method of treating systemic lupus erythematosus in a human patient comprising administering to the patient an effective dose of a CD1d blocking antibody and a second therapeutic agent for treatment of systemic lupus erythematosus, wherein the second therapeutic agent is a non-steroidal anti-inflammatory drug, corticosteroid, immunomodulating drug and/or an anticoagulant.

Claim 22 complies with 35 U.S.C. § 112, first paragraph, because the specification provides sufficient written description with respect to an immunomodulating drug. An objective standard for determining compliance with the written description requirement is, “does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed.” *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989). Under *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991), to satisfy the written description requirement, an applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, and that the invention, in that context, is whatever is now claimed. An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations. *Lockwood v. American Airlines Inc.* (CA FC) 41 USPQ2d 1961 (at 1966).

Furthermore, what is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231 USPQ at 94; *See also, Capon v. Eshhar*, 418 F.3d 1349, 1357, 76 USPQ2d 1078, 1085 (Fed. Cir. 2005)(“The ‘written description’ requirement must be applied in the context of the particular invention and the state of the knowledge.... As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution.”). If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met. See, e.g., *Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116; *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (stating “the description need not be in *ipsis verbis* [i.e., “in the same words”] to be sufficient”).

In the instant case, the Examiner appears to take issue with the claim element "immuno-modulating drug". In particular, the Examiner asserts that "[t]he species of immunomodulating drugs disclosed in the instant specification have different structures and mechanisms of action. The specification does not disclose a structure/function relationship for immunomodulating drugs that modulate some aspect of an immune response that alleviate symptoms of lupus and work in tandem with CD1d-blocking antibody." (Office Action, page 3, paragraph 4). The Examiner further asserts that the specification does not disclose any other drugs besides the "six drugs that are immuno-modulating drugs" (i.e., methotrexate, cyclosporine, chloroquine, hydroxychloroquine, azathioprine and cyclophosphamide). OA, p. 3, last paragraph.

It is respectfully pointed out that the Examiner's concern with structure to function relationship is misplaced and incorrect. First, the structure to function correlation of an immunomodulatory agent is sufficiently disclosed. Second, one of ordinary skill in the art would understand the plain meaning of immunomodulatory agent in terms of treating lupus. Third, the specification provides additional examples beyond the purported six on which the Examiner focuses. Fourth, one of skill would recognize what are immunomodulating agents based on conventional knowledge in the relevant art.

Indeed with respect to the foregoing points, the specification explicitly teaches (page 2, paragraph 0006):

A variety of biologic agents are under investigation as potential treatments for SLE. These products are designed to specifically interfere with immunologic processes, including T cell activation; T cell-B cell collaboration; production of antidouble-stranded DNA antibodies; deposition of anti-double-stranded DNA antibody complexes; complement activation, and immune complex deposition and cytokine activation and modulation. More aggressive interventions include gene therapy and stem cell transplantation. **Immunomodulatory agents recently tested include thalidomide, ASIO1,2' chlordeoxyadenosine, mycophenolate mofetil, and bindarit.** Additional pharmaceutical treatments include the mild androgen dehydroepiandrosterone, selective estrogen receptor modulators, and the prolactin inhibitor, bromocriptine. (emphasis added)

The specification, at least as illustrated by the foregoing disclosure, provides various aspects of the immune reaction that can be modulated by immunomodulatory agents, including T cell activation, T cell-B cell collaboration or complement activation to name a few. One of skill would recognize and readily be able to identify

immunomodulatory agents that function in the preceding manner. Furthermore, upon reading the entirety of the instant specification, one of ordinary skill in the art would comprehend that "immunomodulatory agents" are in the context of treating lupus. In addition, as the exemplary disclosure above makes clear, the Examiner has overlooked and clearly missed entire portions of the specification which disclose and characterize immunomodulatory agents (i.e., more than the six the Examiner purports). Moreover, the Examiner has clearly failed to appreciate immunomodulatory agents which are known and conventional in the relevant art (e.g., thalidomide, ASIO1,2' chlordeoxyadenosine, mycophenolate mofetil, and bindarit). Furthermore, with a basic understanding of immunomodulatory agents known in the art, and upon reading the instant disclosure, one of ordinary skill in the art would immediately appreciate the claimed method of treating lupus with the CD1d blocking antibody and immunomodulatory agent.

In sum, claim 22 is sufficiently described in the instant specification. The Examiner has erred in the rejection and it is respectfully requested that the rejection be reversed.

**THE EXAMINER ERRED IN REJECTING CLAIMS 15-26 AS UNPATENTABLE
UNDER 35 U.S.C. § 103(a)**

The Examiner has rejected claims 15-26 as being obvious over a combination of five to seven references applied in four separate rejections under 35 U.S.C. § 103(a), each of which is addressed in turn below.

In order to establish a prima facie case of obviousness, the Examiner must demonstrate that the prior art (i) teaches or suggests every claim limitation, (ii) provides a motivation to combine (or modify) the teachings of the selected references, and (iii) provides a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991); MPEP § 2143. Rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, "there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness". *KSR Int 'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1741 (2007) (quoting *In re Kahn*, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006)). Thus, in order to establish a prima facie case of obviousness, it is necessary for the Examiner to identify the reasons why a person of ordinary

skill in the art would have combined the prior art elements in the manner claimed. Moreover, it is improper for the Examiner to use the claimed invention as a blueprint for abstracting individual teachings from the cited references, as this is not a proper basis for an art-based rejection. *Ashland Oil Inc. v. Delta Resins & Refractories Inc.*, 776 F2d 281, 297 (Fed. Cir. 1985).

The Examiner has abstracted together individual teachings from the cited references, which simply cannot be combined to arrive at the claimed subject matter. Applicants respectfully submit that there is no motivation to combine the teachings cited by the Examiner in a manner that results in Applicant's claimed invention.

2. Claims 15-20 and 23-26 are patentable under 35 U.S.C. 103(a) over Amano, in view of Kotzin, Zeng, Blumberg and Hughes.

The Applicants' claims are directed to a method of treating lupus in a human patient by administering CD1d blocking antibody. In the Office Action mailed February 06, 2008 (hereinafter "Office Action"), the Examiner provides a combination of various disclosures of the cited references Amano, Kotzin, Zeng, Blumberg and Hughes, but without any real cogent basis as to why one of skill would be led to combine the various individual teachings to arrive at the claimed process.

a) Summary of Cited Art

The primary reference, Amano, discusses T cells that carry the $V_{\beta}9$, $V_{\alpha}4.4$ T cell receptor (TCR). More particularly, Amano discloses that CD1 expression defines subsets of follicular and marginal zone B cells in the spleen and that such B cells may be a critical site for interactions between T and B cells for certain subsets of microbial antigens. The reference does not teach or suggest treatment of lupus as in the claimed subject matter. A more complete discussion of this and additional references can be found in the following explanations of why the claimed inventions would not have been obvious from the combined prior art.

i. *Amano does not render the claimed invention obvious*

The Examiner asserts in salient part the following: (1) Amano allegedly teaches that the interaction between anti-CD1 T cells and B cells leads to mutual activation of both cell types that results in hypergammaglobulinemia and systemic autoimmunity via CD1 cross-linking resulting in secretion of IgM and IgG. (Office Action, page 4, last paragraph); (2) Amano allegedly teaches that T cell proliferation of a CD1-restricted T cell clone in response to CD1-transfected B cells could be

blocked by use of an anti-CD1d monoclonal antibody ("mAb"). *Id.*; and (3) Amano allegedly teaches that CD1 appears to be recognized by a T cell subset which has a restricted TCR repertoire that is made up predominantly of an invariant rearrangement of the $V_{\alpha}14J_{\alpha}281$ associated with $V_{\beta}2$, $V_{\beta}7$, $V_{\beta}8$ receptors, and that T cells that do not express NK1.1 marker or $V_{\alpha}14$ TCR are able to recognize CD1 on syngenic antigen presenting cells. The Examiner also asserts that Amano does "not teach the claimed method of treating pathogenic polyclonal B cell activation or class switching, including that resulting in lupus (SLE), in a human patient, comprising administering a CD1 [CD1d] blocking agent that is an antibody, including a monoclonal antibody." Office Action, p. 5.

The Examiner essentially provides a listing of various alleged disclosures by Amano without any conclusion or rationale as to how such assertions, even if correct, would render the claimed invention unpatentable. Indeed, the teachings whether alone or combined with the other cited art cannot lead to the claimed invention, because Amano do not provide a reasonable expectation of success or an adequate level of predictability to administer anti-CD1d antibody to treat lupus.

The Amano reference provides an *in vitro* experimental model which is an absolutely artificial system that uses one T cell line that is genetically engineered to express a receptor that recognizes CD1. Put another way, 100% of the cells have a receptor that they would not normally have and which does not occur in a native *in vivo* system (e.g., subject or patient). Furthermore, Amano demonstrate that this T cell line proliferates in response to exposure to the CD1d expressed on the surface of B cells. In contrast, with lupus, it is the proliferation of B cells and the secretion of antibodies coupled with the later switching of antibody class that form the hallmarks of the disease. None of these phenomena are demonstrated or even suggested by the Amano reference. The demonstration of inhibition of T cell proliferation with an anti-CD1d antibody in Amano of does not teach or suggest the use of this antibody to treat lupus since there is no demonstration or suggestion that NKT cells, let alone T cells, can stimulate the proliferation of B cells. With no demonstration or suggestion that this interaction occurs, there can be no suggestion that it is beneficial to block the interaction to prevent B cell proliferation.

Additionally, at the time of invention, it was known that deletion of $CD4^{+}$ T cells, a much larger cell population than NKT cells, effectively treats disease. Knowing this fact, a person of skill would not be led to believe that lupus could be treated by targeting a small cell population like the 3-

4% in mice or the 0.1% in humans that NKT cells represent out of the total T cell population. Instead, a person of skill would retain the commonly held belief that another, larger cell population was involved in the etiology of lupus and would not look upon the results of Amano with any expectation of success, particularly since Amano does not demonstrate that NKT cells or even T cells stimulate B cells to proliferate, secrete antibodies and to later undergo class switching.

The alleged interaction between anti-CD1 T cells and B cells that leads to mutual activation of both cell types with resultant hypergammaglobulinemia and systemic autoimmunity is attributed by Amano to Zeng and will be addressed in section 1.a.ii. below.

Moreover, even assuming *arguendo* that Amano when combined with Zeng suggest that CD1d is implicated in SLE, this would not rise to the level of suggesting that blocking CD1d would be effective in treating the disease.

ii. *Zeng in view of Amano does not render the claimed invention obvious*

As with Amano, the secondary reference Zeng also discusses an artificial system, this time consisting of a transgenic mouse model where all the T cells carry the $V_{\beta}9$, $V_{\alpha}4.4$ T cell receptor (TCR). Furthermore, Zeng teaches that subsets of transplanted transgenic T cells that recognize CD1 may either **induce** or **prevent** murine lupus in recipient mice, thus explicitly outlining the unpredictability associated with T cell involvement. Zeng also discloses using anti-CD1d antibody for isolation of an IgM fraction from anti-CD1 hybridoma supernatants.

Further unpredictability of the references is demonstrated by the fact that when the $V_{\beta}9$, $V_{\alpha}4.4$ receptor from Amano is expressed as a transgene in Zeng, two types of T cells were created. One transgenic mouse had T cells with single positive cells (CD4+CD8- or CD4-CD8+), while a second transgenic mouse had double negative cells (CD4-CD8-) like those in Amano. Zeng found that the injection of the double negative cells were protective of disease, while the single positive cells, which do not correspond to the original cell type, caused a disease phenotype. A person of skill in the art when presented with this data would be confused as to the relevance of the interaction between T cells and B cells since these are two examples of T cells with the same receptor producing contradictory results in the same model.

In contrast, Applicant used a non-genetically engineered, hereditary model of lupus where NKT cells are present at about 3-4% of CD4+ T cell populations. Applicant further demonstrated

that these CD1 reactive cells (NKT cells) are involved in mediating lupus and that blocking CD1d ameliorates disease. Furthermore, in contrast to the artificial model provided in Amano and Zeng, Applicant shows that by administering anti-CD1d antibody, onset of disease (e.g., proteinuria) is delayed and survival is prolonged. Without an understanding that NKT cells mediate disease, one would simply not apprehend the disclosure of Amano and Zeng (below) to suggest with any reasonable predictability the treatment of lupus by administration of anti-CD1d antibody. Furthermore, in the transgenic mice, the receptor for CD1d is found on all the T cells, while conversely it is only present on about 0.1% of the greater T cell population (i.e., NKT cells) in humans. As such, at the time of invention, one would not reasonably extrapolate from the results seen with the universal expression of the CD1d receptor in Amano and Zeng, to the treatment of humans with an anti-CD1d antibody with any expectation of success given the exceedingly small prevalence of NKT cells in humans.

iii. *Kotzin alone or in combination with any of the cited prior art does not render the claimed invention obvious*

The tertiary reference Kotzin is a review article which discusses production of IgG autoantibody production in SLE. The Examiner asserts that Kotzin allegedly teaches IgG autoantibody production in SLE by clonal expansion of somatically mutated anti-DNA antibody-producing B cells. Office Action, p. 5. Furthermore, the Examiner asserts that Kotzin teaches that IgG antibodies to ds-DNA appear to play a role in immune complex glomerulonephritis of SLE. In sum, it is unclear what the Examiner's reasoning is in citing Kotzin and how or for what reason one of skill at the time of invention would view Kotzin as suggestive to administer any antibody, save anti-CD1d, to treat lupus. Here, as with Amano and Zeng, the Examiner merely recites every purported disclosure of the cited reference without establishing any nexus between the teachings or articulating a reasonable basis for arriving at the claimed invention.

The additional references Blumberg and Hughes, respectively, discuss cell selection/identification using various CD1 antibodies and the concept of humanizing monoclonal antibodies generally, and therefore, do not provide a rationale for using the anti-CD1d antibodies in treatment of lupus. The references do not cure the deficiencies of the other references used in the rejection.

b) Examiner's Rationale

The Examiner's conclusion is recited as follows (OA, page 7):

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have used the anti-CD1d mAB taught by Zeng *et al* or Amano *et al* the anti-CD1d antibodies taught by Blumberg *et al* to block CD1 recognition by T cells as taught by Amano *et al* by administration of antibodies to human patients with SLE, and hence to treat pathogenic polyclonal B cell activation or class switching taught by Kotzin *et al*, including with humanized versions of the said antibodies as taught by Hughes for human patients with autoimmune diseases, and including by the intravenous (IV) route of administration of T cells by Zeng *et al*. One of ordinary skill in the art at the time the invention was made would have been motivated to do this to treat pathogenic polyclonal B cell activation or class switching in a patient with SLE with a reasonable certainty of success because...[OA, pages 7-8 omitted].

In reviewing the foregoing recitation, as well as the omitted sections provided in two full pages (OA, pages 7-8), there is no reasoned basis or sufficient rationale provided as to why the recited disclosures should be combined, and even if combined how one of skill would arrive at the claimed invention. It is apparent that the Examiner is improperly abstracting individual teachings of the cited references based on the Applicant's invention. In the explanation of what the references teach and what would have been obvious, the Examiner provides an inventory of virtually every disclosure in the cited references. OA, pages 4-7. This extensive listing actually reinforces the non-obviousness of the claimed inventions. As to the claimed methods, it appears the Examiner is primarily relying on the Amano, Zeng and Kotzin references; a summary of the alleged teachings of these references is provided above.

The Examiner asserts that Amano does not teach treating pathogenic polyclonal B cell activation or class switching, through administration of a CD1 blocking antibody. OA, page 5, second full paragraph. The Examiner also asserts that Kotzin discloses clonal expansion of B cells producing IgG autoantibody, which mimics normal T cell dependent response to foreign antigen, involving common mechanisms of affinity maturation and IgM to IgG class switching. OA, page 5, third paragraph. The Examiner appears to be alleging that because Amano and Zeng allegedly disclose T cell-B cell interaction through CD1, and Kotzin allegedly discloses involvement of B cell class switching in lupus, then it would have been obvious to treat lupus by administering anti-CD1d

antibody to a human subject. However, such construction is necessarily built on improper hindsight reasoning, because at the time of invention the prior art held the misconstrued view that CD4⁺ helper T cell involvement induced B cell activation (or proliferation) or class switching. (*infra*, Swain, SL, 1983; Swain et al. 1984; Wofsy and Seaman, 1985). Moreover, none of the references actually show any treatment of lupus and one would not have any expectation of success from the combined teachings of the references.

As further explained in the discussion below, the combination of references is both improper and would not have led one of ordinary skill in the art at the time of invention to consider CD1d antibody therapy for lupus.

c) Combination of Cited References Does Not Lead to Claimed Invention

The claimed subject matter is not rendered obvious by the cited prior art because at the time of the invention, the collective view held that it was the interaction of CD4⁺ helper T cells with B cells that induced B cells to proliferate, secrete autoantibodies and undergo class switching from IgM to IgG. See Swain SL. Immunol. Rev. 74:129, 1983; and Swain et al. J. Immunol. 132:1118-23, 1984. This teaching away was reinforced by the demonstration that depletion of CD4⁺ helper T cells with anti-CD4 mAb ameliorated lupus in the hereditary murine model NZB/NZW F1. See Wofsy D, and Seaman SE. J. Exp. Med., 161:378-91, 1985.

In other words, CD1d and NKT cells as disclosed in the instant specification were not believed to be involved in the etiology of lupus. Thus, the prior art taught away from Applicant's discovery that CD1d and NKT cells are important effectors in the etiology of lupus.

The claims are directed to a method of treating a human patient by administering a CD1d blocking antibody, which inhibits pathological polyclonal B cell activation or class switching (e.g., claim 16). The fact of the matter is that none of the cited references show or suggest treating lupus by administering CD1 blocking antibody. The Examiner misapprehends either what is encompassed by the instant claims or what Amano – the primary reference – teaches. For example, the Examiner states (emphasis added):

Applicant argues that Zeng does not teach the role of NK T cells in lupus because the experiments (Amano and Zeng) did not study NK T cells, the arguments based on upon the assertion that the transgenic mouse model used carried the V α 4.4 TCR, but that NK T cells express V α 14 TCR.

Applicant further argues that it would not have been predictable that spontaneous lupus found in NZB/NZW mice that express V α 14 TCR (Applicant's model) could be treated with an anti-CD1d antibody. **However, Amano *et al* teach that the V α 4.4 T cell clone proliferates in response to CD1-transfected B cells, that this proliferation is blocked by an anti-CD1d mAb, and that this T cell clone can induce lupus.**

More particularly, as underscored by the highlighted portion above, and acknowledged by the Examiner, Amano did not demonstrate that a T cell clone could induce the proliferation of B cells, B cell activation with the resultant secretion of antibodies or class switching of antibody type from IgM to IgG. See page 1714, 2nd column, 1st paragraph under heading *T cell recognition of CD1 is not associated with β_2m* . Instead, Amano demonstrated the opposite result by showing that a transgenically derived T cell clone, V β 9/V α 4.4, vigorously proliferated in response to a CD1-transfected B cell line.

In addition, there is no reason to generalize that one CD1d recognizing T cell clone that induces lupus shows or implies that all CD1d recognizing T cells will induce lupus. In fact, Zeng teaches that CD1d recognizing transgenic T cells can induce or suppress lupus depending on the tissue of origin and the cytokine secretion pattern. Furthermore, the finding that one CD1d recognizing T cell clone can induce lupus does not teach whether the considerably more numerous MHC recognizing CD4⁺ T cells will induce lupus also. Lupus induction by these MHC recognizing T cells would not be expected to be blocked by anti-CD1d monoclonal antibody. Therefore, Zeng does not teach that all CD1d recognizing T cells induce lupus or that MHC recognizing T cells do not induce lupus or that any T cell that induces lupus can be blocked with an anti-CD1d monoclonal antibody or that spontaneously occurring lupus can be blocked with an anti-CD1d monoclonal antibody. There was no reason to expect at the time of the paper of Zeng that the cellular and molecular mechanisms that cause lupus induced by CD1d recognizing transgenic T cells derived from a single T cell clone are the same or similar to the mechanisms that cause spontaneous lupus in mice or humans.

A key finding by the Applicant, which provides the reasoning to treat lupus with antibodies against CD1d, is that NKT cells mediate B cell activation and class switching, thus resulting in lupus. Both Amano and Zeng are limited to the convention in the prior art that conventional T cells

are involved in the etiology of lupus. Declaration of Dr. Samuel Strober, paragraphs 4-5 (hereinafter "Declaration"). In contrast, Applicant discovered that it is the NKT cell mediation of antibody production and isotype switching that results in disease. For example, as discovered by Applicant, incubation of conventional T cells with B cells does not result in significantly increased secretion of IgM or IgG isotypes as compared to cultures of B cells alone. Declaration, paragraph 5.

However, co-culturing NKT cells with splenic B-1 or marginal zone B cells secreted markedly increased amounts of IgM and IgM anti-dsDNA antibodies. *Id.*

Amano does not teach about the interaction of CD1 and NKT cells because the cell line used for the experiments was a T cell line that was engineered to express the $V_{\beta}9$, $V_{\alpha}4.4$ TCR. In contrast, the NKT cells from NZB/W mice, as described in the instant specification, express $V_{\alpha}14J_{\alpha}18$. The use of an engineered cell line that does not have the correct TCR is further evidence that the model of Amano and Zeng would not be predictive of success in administering anti-CD1d antibodies in treatment of SLE in humans.

Zeng does not teach the role of NKT cells in lupus, because the experiments did not study NKT cells. Instead, a transgenic mouse model was used where all of the T cells carried the $V_{\beta}9$, $V_{\alpha}4.4$ TCR. NKT cells, on the other hand, express a unique and invariant TCR, $V_{\alpha}14J_{\alpha}18$. Furthermore, the Zeng's teachings were limited to *in vitro* demonstrations that transgenically derived T cells expressing the CD1d TCR transgenes could stimulate the proliferation of non-transgenic B cells. However, importantly, in the transgenic animal model these T cells did not cause the development of lupus. See Zeng, page 527, column 1, first paragraph. Thus, contrary to the Examiner's assertion, there would not be an expectation of success in the claimed method of treatment, particularly since in humans only 0.1% of the T cells are NKT cells as opposed to 100% of the cells in the Zeng model. Furthermore, at the time of invention, NKT cells were believed to be protective and not causative for lupus.

Only when Zeng transferred T cells from transgenic mice to irradiated *nu/nu* host mice were they able to induce lupus. Even here, only certain subsets of T cells induced disease notwithstanding the fact that 100% of the T cells expressed the CD1d TCR transgenes. Normally, in mice, the CD1d TCR is only found on NKT cells which make up approximately 3-4% of the greater T cell family.

More specifically, Zeng transplanted bone marrow with or without added sorted T cells. T cells were sorted based on CD4 and CD8 expression and were also classified as to organ source. When bone marrow from single positive (SP, i.e., CD4⁺ or CD8⁺) transgenic mice was injected into irradiated hosts, 9 of 20 developed ascites, and 15 of 20 developed proteinuria and anti-double stranded DNA antibodies; the three markers for lupus. See page 527, column 2, last paragraph and Table 2. Bone marrow from double negative (DN, i.e., CD4⁻ and CD8⁻) transgenic mice did not induce disease in 12 of 12 host mice. See page 528, column 1, lines 8-11 and Table 2. SP bone marrow admixed with splenic SP sorted T cells induced disease in 6 of 6 mice. See page 528, column 1, lines 3-8 and Table 2. The addition of splenic DN sorted T cells accelerated disease induction when admixed with SP bone marrow. See page 529, column 1, lines 20 – column 2, lines 1-2. Bone marrow from nontransgenic nude mice admixed with splenic SP T cells induced two of the three hallmarks of lupus, as did non-transgenic nude mouse bone marrow admixed with splenic DN T cells. See page 529, column 1, second paragraph and Table 2. Bone marrow from nontransgenic nude mice admixed with CD4⁺ or CD8⁺ sorted T cells induced two of the three hallmarks of lupus. See Table 2.

Mixing experiments of bone marrow cells suggested bone marrow from DN mice may reduce the incidence of disease when added to SP bone marrow as 2 of 8 mice developed all three hallmarks of lupus compared to 9 or 15 of 20 mice developing the individual hallmarks of lupus in mice that received only SP bone marrow. See page 529, column 2, section entitled CD4⁻CD8⁻ T Cells from Transgenic Marrow Prevent Lupus and Table 3. Further experiments demonstrated the importance of the organ source of DN T cells with bone marrow derived DN T cells suppressive and splenic DN T cells causative of disease. See Table 3. Zeng concluded that “[t]he inhibitory activity of the DN transgenic BM cells was related to the presence of the transgenes, since substituting 2.5 x 10⁶ BM cells from nontransgenic BALB/C mice failed to inhibit the induction of lupus abnormalities by the SP transgenic BM cells.” See page 529, column 2, lines 25-30.

Unable to correlate the induction of disease with CD4 or CD8 status, Zeng next studied the cytokine expression pattern of the transgenic T cells and discovered that bone marrow DN T cells had the Th2 secretion pattern with high levels of IL-4 and relatively low levels of IFN-γ and IL-2. In contrast, SP, double positive (CD4⁺ and CD8⁺), and splenic DN T cells exhibited the Th1 cytokine

expression pattern with high levels of IFN- γ and IL-2 and relatively low levels of IL-4. See page 529, column 2, section entitled: Cytokine Profile of Transgenic T Cells That Induce or Prevent Lupus.

In sum, at the time of invention there would be no reasonable expectation of success, for one of skill to treat lupus using anti-CD1d antibody, because one of skill would have recognized at the time of invention that Amano and Zeng provide an artificial system that does not reflect what actually occurs (i.e., Applicants' discovery of the unexpected result where NKT cells activate CD1 on B cells sufficiently for the development of a pathology) and is not a representative system from which a person of art could reasonably extrapolate to the treatment of humans.

Moreover, one of ordinary skill in the art at the time of invention needed to consider the entire teachings of the cited references as a whole and not extract isolated portions. As such, the preponderance of the teachings of the Amano and Zeng would be more confounding than predictive and would not have led to the instantly claimed methods.

In particular, one of skill would not have been motivated to combine the aspects from the studies presented by Amano and Zeng together or with the other cited references because it would be known that 1) normal mice only express CD1d TCR on NKT cells and not on T cells, 2) NKT cells only represent 3-4% of the greater T cell population in mice and 0.1% in humans, 3) the T cells used for the experiments were isolated from transgenic mice where 100% of the T cells express CD1d TCR, 4) the transgenic mice do not develop disease, 5) the recipient mice were lethally irradiated to condition them for accepting the donor cells, 6) disease was only induced in some recipient mice, 7) no correlation was found for T cell markers and disease induction, and 8) Zeng specifically concluded that the presence of the transgenes in bone marrow T cells is inhibitory for the development of lupus. Therefore, one of skill would not be led to conclude that the combination of the results of Amano and Zeng are applicable to arriving at the claimed inventions, because Amano and Zeng do not teach or suggest that administration of anti-CD1d antibody could treat lupus.

Rather than finding motivation to combine references, the person of ordinary skill in the art would espouse the conventional view that CD4⁺ helper T cells are required for activation of B cells because CD4⁺ helper T cells are more prevalent than NKT cells and it was known that the deletion

of CD4⁺ helper T cells by anti-CD4 antibodies would ameliorate disease in hereditary murine models in a simple and direct manner.

Moreover, even if one CD1d recognizing T cell clone could induce lupus there is nothing in Amano or Zeng to show or imply that all CD1d recognizing T cells will induce lupus, notwithstanding the fact that in Zeng transgenic T cells with the same receptor can induce or suppress lupus depending on the tissue of origin and the cytokine secretion pattern. Furthermore, the finding that one CD1d recognizing T cell clone can induce lupus does not teach whether the considerably more numerous MHC recognizing CD4⁺ T cells will induce lupus also. Lupus induction by these MHC recognizing T cells would not be expected to be blocked by anti-CD1d monoclonal antibody. Therefore, Zeng does not teach that all CD1d recognizing T cells induce lupus or that MHC recognizing T cells do not induce lupus or that any T cell that induces lupus can be blocked with an anti-CD1d monoclonal antibody or that spontaneously occurring lupus can be blocked with an anti-CD1d monoclonal antibody. There was no reason to expect at the time of the paper of Zeng that the cellular and molecular mechanisms that cause lupus induced by CD1d recognizing transgenic T cells derived from a single T cell clone are the same or similar to the mechanisms that cause spontaneous lupus in mice or humans.

Applicants now have clinical data to further support the discovery that blocking CD1d TCRs with antibody reduces the secretion of anti-double stranded DNA antibodies, a marker for lupus. *See, e.g., Declaration, paragraph 5.* Normal CD-19⁺ B cells do not spontaneously secrete IgM or IgA in culture. When normal NKT cells are added, IgM and IgA secretion is detected. In contrast, CD-19⁺ B cells from lupus patient spontaneously secrete considerable amounts of IgM, IgA and IgG. When co-cultured with NKT cells, B cells increased antibody production 2-10 fold. When anti-CD1d antibody was added anti-double stranded DNA IgG production was significantly reduced.

In sum, the Examiner erred in the rejection of the instant claims. The Examiner has failed to establish a prima facie case of obviousness, because the Examiner has failed to establish a reasonable basis for combining the multiple cited references and the Examiner has failed to establish a reasonable expectation of success based on knowledge in the relevant art at the time of invention.

For the reasons described above, claims 15-20 and 23-26 are not obvious under 35 U.S.C. § 103 and are therefore patentable. Therefore, the Examiner has erred in the rejection and the Examiner's obviousness rejection should be reversed.

3. Claims 21 and 22 are patentable under 35 U.S.C. 103(a) over Amano, in view of Kotzin, Zeng, Blumberg, Hughes and Merck Manual.

Claim 21 is dependent from claim 15 and is directed to a method of treating a human subject for lupus erythematosus by administering a second therapeutic agent for treating SLE. Claim 22 further defines options for such second therapeutic agents.

For the reasons provided above (Section VII(B)(1)), the combined teachings of the cited references do not teach or suggest the claimed subject matter of independent claim 15. The Merck Manual does not cure the deficiencies in the grounds of rejection as presented. As such, dependent claims which require claim elements of intervening claims are also necessarily patentable.

Therefore, the Examiner's obviousness rejection should be reversed.

4. Claims 15-26 are patentable under 35 U.S.C. 103(a) over Amano, in view of Kotzin, Zeng, the '453 patent, Blumberg, and Hughes.

The Examiner submits a rejection for claims 15-26 based on the above captioned references. In the rejection, the Examiner repeats the purported teachings of Amano, Kotzin, Zeng, Blumberg and Hughes consonant with what is discussed above in Sections VII(B)(1)-(2). In addition, the Examiner asserts that the '453 patent discloses that a decrease in V α 14 NK T cells is closely associated with the onset of lupus in a murine model. OA, page 14, paragraph 2. This association contradicts the Examiner's previous assertions regarding the significance of Amano as presented in the Office Action at page 12, bottom paragraph. For if T cell proliferation can be blocked by the use of an anti-CD1d antibody a person of skill would reasonably conclude that the use of an anti-CD1d antibody would be contraindicated in the treatment of lupus because, administration of the antibody would be expected to further depress NKT cell number by blocking their proliferation and thereby accelerate the development or exacerbate the symptoms of the disease.

In sum, the Examiner has failed to establish a prima facie case of obviousness as set forth above in the preceding sections. In addition, the disclosure of the '453 patent contradicts the purported teaching of Amano and Zeng and therefore does not provide a reasonable basis for

combining these multiple cited references. Furthermore, the Examiner has failed to establish a reasonable expectation of success based on knowledge in the relevant art at the time of invention.

For the reasons described above, claims 15-26 are not obvious under 35 U.S.C. § 103 and are therefore patentable. Therefore, the Examiner has erred in the rejection and Examiner's obviousness rejection should be reversed.

5. Claims 15-26 are patentable under 35 U.S.C. 103(a) over Amano, in view of Kotzin, Zeng, the '453 patent, Blumberg, Hughes, and Merck Manual.

The Examiner rejects claims 15-26 based on the combined disclosure of the previously cited seven references listed in the above three rejections. This rejection adds no new arguments or references.

Thus, for the reasons described above, the cited references do not disclose or suggest a method of treating systemic lupus erythematosus in a human patient with a CD1d blocking antibody as discussed in Sections VII(B)(1)-(3) above. Furthermore, the Examiner has failed to establish a reasonable expectation of success based on knowledge in the relevant art at the time of invention. Therefore, the Examiner has failed to establish a prima facie case of obviousness and the rejection of claims 15-26 under 35 U.S.C. § 103(a) should be reversed.

CONCLUSION

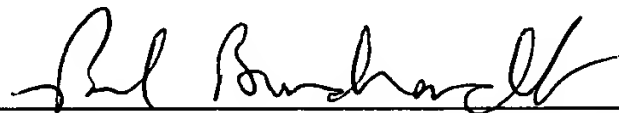
For the reasons stated above, claims 15-26 are patentable over the prior art of record, and the rejection of claim 22 under 35 U.S.C. § 112, written description, is improper. Appellants respectfully request the Board to reverse the Examiner's rejections with instructions to allow the claims.

The Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to Deposit Account No. 23-2415 (Attorney Docket No. 31580-702.201).

Respectfully submitted,

Date: November 16, 2009

By:



Anie Roche, Ph.D. (Reg. No. 50,512)

Paul Borchardt, Ph.D. (Reg. No. 53,999)

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VIII. CLAIMS APPENDIX

1-14. (Canceled)

15. (Previously Presented) A method of treating systemic lupus erythematosus in a human patient comprising administering to said patient an effective dose of a CD1d blocking antibody, wherein said effective dose treats said systemic lupus erythematosus in said human patient.

16. (Previously Presented) The method of Claim 15 wherein said administration inhibits a pathologic polyclonal B cell activation or class switching.

17. (Previously Presented) The method according to Claim 15, wherein said antibody is a monoclonal antibody.

18. (Previously Presented) The method according to Claim 17, wherein said monoclonal antibody is a human or humanized antibody.

19. (Previously Presented) The method according to Claim 17, wherein said monoclonal antibody specifically binds to human CD1d.

20. (Previously Presented) The method according to Claim 15, wherein said administration is by intravenous injection.

21. (Previously Presented) A method according to Claim 15, further comprising administering to said patient a second therapeutic agent for the treatment of systemic lupus erythematosus.

22. (Previously Presented) The method of Claim 21, wherein said second therapeutic agent is a non-steroidal anti-inflammatory drug, corticosteroid, immunomodulating drug, and/or an anticoagulant.

23. (Previously Presented) A method of treating systemic lupus erythematosus in a human patient comprising administering to said patient an effective dose of a CD1d blocking antibody, wherein said effective dose treats said systemic lupus erythematosus in said human patient and inhibits a pathologic polyclonal B cell activation or class switching.

24. (Previously Presented) A method of treating systemic lupus erythematosus in a human patient comprising administering to said patient an effective dose of a CD1d blocking antibody, wherein said effective dose treats said systemic lupus erythematosus in said human patient and delays the onset of proteinuria.

25. (Previously Presented) The method according to Claim 24, wherein said administering to said patient an effective dose of a CD1d blocking antibody reduces the levels of serum IgG and anti-dsDNA IgG.

26. (Previously Presented) The method according to Claim 24, wherein said administering to said patient an effective dose of a CD1d blocking antibody prolongs survival of said patient.

IX. EVIDENCE APPENDIX

This appendix contains three copies of Swain SL, T Cell Subsets and the Recognition of MHC Class, Immunol. Rev. 74:129-142, 1983; Swain SL et al., Monoclonal Antibody to L3T4 Blocks the Function of T Cell Specific for Class 2 Major Histocompatibility Complex Antigens, J. Immunol. 132:1118-1123, 1984; and Wofsy D, et al., Successful Treatment of Autoimmunity in NZB/NZW F₁ Mice with Monoclonal Antibody to L3T4, J. Exp. Med., 161:378-391, 1985, that were submitted on April 28, 2006 in an Information Disclosure Statement pursuant to 37 C.F.R. §1.97 and 37 C.F.R. §1.98. The articles were entered into the record on July 17, 2006 as indicated by the initialed form PTO/SB/08A.

This appendix further contains three copies of a declaration by Dr. Samuel Strober that was submitted on October 30, 2007 pursuant to 37 C.F.R. §1.132 and entered into the record as indicated on page 2 of the Non-Final Office Action mailed February 6, 2008.

AMENDED APPELLANT'S BRIEF UNDER 37 C.F.R. §41.37
U.S. Appl. No. 09/844,544; Filed on November 16, 2009
Docket No. 31580-702.201

X. RELATED PROCEEDINGS APPENDIX

None.



FILED ELECTRONICALLY ON OCTOBER 30, 2007

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Attorney Docket No. 31580-702.201

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Inventors: Defu Zeng et al.)	
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<i>Blocking CDI-Mediated Interactions</i>)	

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DECLARATION PURSUANT TO 37 CFR §1.132

Dear Sir or Madam:

I, Dr. Samuel Strober, M.D., do hereby declare as follows:

1. I am a Professor of Medicine in the Department of Medicine, Division of Immunology, Stanford University School of Medicine, Stanford, CA. I received my M.D. from Harvard Medical School, Boston, Magna Cum Laude in 1966. I have over thirty years of experience in immunology.
2. I am familiar with the prosecution history of the above-identified patent application and the pending obviousness issues.
3. I am submitting this declaration to show that the use of an anti-CD1 antibody to treat lupus is not obvious over the prior art cited by the Examiner. The references used by the Examiner include Zeng et al., Subsets of transgenic T cells that recognize CD1 induce or prevent murine lupus; Role of cytokines. J. Exp. Med. 187:525-526, 1998, and Amano et al., CD1 expression defines

subsets of follicular and marginal zone B cells in the spleen: β_2m -dependent and independent forms. J. Immunol., 161: 1710-1717, 1998. The Examiner states that the results of Zeng et al. in combination with Amano et al. makes obvious the invention of the use of anti-CD1 mAb to treat lupus.

4. Both Zeng and Amano are the result of experiments conducted in my laboratory at Stanford University. Zeng does not teach the role of NKT cells in lupus because the experiments did not study NKT cells. Instead, a transgenic mouse model was used where all of the T cells carried the $V_{\beta}9$, $V_{\alpha}4.4$ T cell receptor (TCR). NKT cells, on the other hand, express a unique and invariant TCR, $V_{\alpha}14J_{\alpha}18$. Similarly, the experiments in Amano do not teach about the interaction of CD1 and NKT cells as the cell lines used were T cells that also express the $V_{\beta}9$, $V_{\alpha}4.4$ TCR. However, the NKT cells from NZB/W mice, as described in my patent application, express $V_{\alpha}14J_{\alpha}18$. Thus, based on Zeng and Amano, it would not have been predictable that spontaneous lupus found in NZB/W mice could be treated with an anti-CD1d antibody.

5. Recent experiments conducted in my laboratory (Takahashi, T. and Strober, S. Natural killer T cells and innate immune B cells from lupus-prone NZB/W mice interact to generate IgM and IgG autoantibodies. In press) demonstrate that the incubation of conventional T cells with B cells does not result in significantly increased secretion of IgM ($p > 0.2$), or IgG isotypes (IgG1, $p = > 0.05$ to 0.5) as compared to cultures of B cells alone. IgG2a was not detected and IgM anti-dsDNA levels were less than 20 U/ml. In contrast, NKT cells co-cultured with splenic B-1 or marginal zone B cells secreted markedly increased amounts of IgM ($p = < 0.0001$ to 0.001) and IgM anti-dsDNA antibodies ($p = 0.0007$ to 0.03) as compared to cultures of B cells alone. Additionally, co-cultures produced significantly increased amounts of IgG1 ($p = 0.0003$ to 0.008) and IgG2a ($p = 0.0003$ to 0.002) that were above 400 ng/ml and 50 ng/ml respectively as well as increased amounts of IgG anti-dsDNA antibodies. The addition of anti-CD1d antibody to co-cultures of purified NKT cells and B cells significantly decrease IgM and IgM anti-dsDNA antibody secretion, $p = 0.004$ to 0.03 for B-1 and $p = 0.003$ to 0.02 for marginal zone B cells. Similarly, the addition of anti-CD1d antibody significantly reduced IgG1 secretion, $p = 0.001$ to 0.01 , and IgG2a secretion, $p = 0.0001$ to 0.001 .

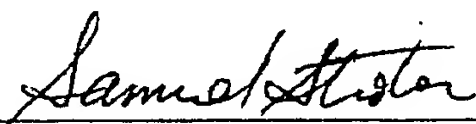
6. Culturing experiments conducted on normal human and lupus patient lymphocytes by my colleague Edgar Engleman at Stanford expound upon my lab's findings and extend the results to humans. Normal human B cells do not spontaneously secrete IgM. When normal NKT cells were

added, however, B cells secrete IgM, but not anti-double stranded-DNA (ds-DNA) IgM or IgG or IgG anti-dsDNA antibodies. Lupus patient B cells, however, spontaneously secrete considerable amounts of IgM (0.05-0.1 ug/ml), IgA (0.05-0.6ug/ml) and IgG (0.1-2ug/ml). Immunoglobulin production is further increased in lupus patient B cells by co-culturing with lupus patient NKT cells where IgG, IgM and IgA production increased about 3-10 fold, 2-4 fold and 2-4 fold, respectively. Moreover, lupus patient NKT cells help lupus patient B cells secrete anti-dsDNA IgG autoantibody, a hallmark of the disease. The addition of anti-human CD1d antibody inhibits production of antibodies by normal and lupus patient B cells. Of particular significance is the discovery that the addition of anti-CD1d antibody significantly reduces anti-dsDNA IgG production. (Figure 1, attached) These data suggest that NKT cells augment antibody production and isotype switching through the recognition of CD1d on B cells.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that making willful false statements and the like are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the applications or any patent issuing thereon.

Dated: October 29, 2007

Respectfully submitted,



Samuel Strober, M.D.

Professor of Medicine

Department of Medicine

Division of Immunology

Stanford University School of Medicine

Stanford, CA



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Attorney Docket No. 31580-702.201

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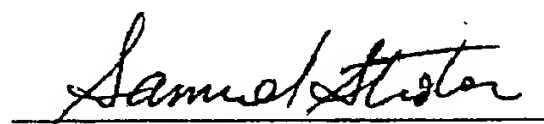
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3. I am submitting this declaration to show that the use of an anti-CD1 antibody to treat lupus is not obvious over the prior art cited by the Examiner. The references used by the Examiner include Zeng et al., Subsets of transgenic T cells that recognize CD1 induce or prevent murine lupus; Role of cytokines. J. Exp. Med. 187:525-526, 1998, and Amano et al., CD1 expression defines

subsets of follicular and marginal zone B cells in the spleen: β_2 m-dependent and independent forms. J. Immunol., 161: 1710-1717, 1998. The Examiner states that the results of Zeng et al. in combination with Amano et al. makes obvious the invention of the use of anti-CD1 mAb to treat lupus.

4. Both Zeng and Amano are the result of experiments conducted in my laboratory at Stanford University. Zeng does not teach the role of NKT cells in lupus because the experiments did not study NKT cells. Instead, a transgenic mouse model was used where all of the T cells carried the $V_{\beta}9$, $V_{\alpha}4.4$ T cell receptor (TCR). NKT cells, on the other hand, express a unique and invariant TCR, $V_{\alpha}14J_{\alpha}18$. Similarly, the experiments in Amano do not teach about the interaction of CD1 and NKT cells as the cell lines used were T cells that also express the $V_{\beta}9$, $V_{\alpha}4.4$ TCR. However, the NKT cells from NZB/W mice, as described in my patent application, express $V_{\alpha}14J_{\alpha}18$. Thus, based on Zeng and Amano, it would not have been predictable that spontaneous lupus found in NZB/W mice could be treated with an anti-CD1d antibody.

5. Recent experiments conducted in my laboratory (Takahashi, T. and Strober, S. Natural killer T cells and innate immune B cells from lupus-prone NZB/W mice interact to generate IgM and IgG autoantibodies. In press) demonstrate that the incubation of conventional T cells with B cells does not result in significantly increased secretion of IgM ($p > 0.2$), or IgG isotypes (IgG1, $p = > 0.05$ to 0.5) as compared to cultures of B cells alone. IgG2a was not detected and IgM anti-dsDNA levels were less than 20 U/ml. In contrast, NKT cells co-cultured with splenic B-1 or marginal zone B cells secreted markedly increased amounts of IgM ($p = < 0.0001$ to 0.001) and IgM anti-dsDNA antibodies ($p = 0.0007$ to 0.03) as compared to cultures of B cells alone. Additionally, co-cultures produced significantly increased amounts of IgG1 ($p = 0.0003$ to 0.008) and IgG2a ($p = 0.0003$ to 0.002) that were above 400 ng/ml and 50 ng/ml respectively as well as increased amounts of IgG anti-dsDNA antibodies. The addition of anti-CD1d antibody to co-cultures of purified NKT cells and B cells significantly decrease IgM and IgM anti-dsDNA antibody secretion, $p = 0.004$ to 0.03 for B-1 and $p = 0.003$ to 0.02 for marginal zone B cells. Similarly, the addition of anti-CD1d antibody significantly reduced IgG1 secretion, $p = 0.001$ to 0.01, and IgG2a secretion, $p = 0.0001$ to 0.001.

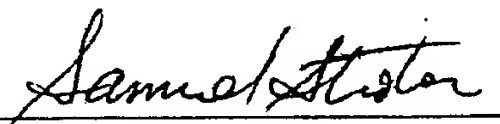
6. Culturing experiments conducted on normal human and lupus patient lymphocytes by my colleague Edgar Engleman at Stanford expound upon my lab's findings and extend the results to humans. Normal human B cells do not spontaneously secrete IgM. When normal NKT cells were

added, however, B cells secrete IgM, but not anti-double stranded-DNA (ds-DNA) IgM or IgG or IgG anti-dsDNA antibodies. Lupus patient B cells, however, spontaneously secrete considerable amounts of IgM (0.05-0.1 ug/ml), IgA (0.05-0.6ug/ml) and IgG (0.1-2ug/ml). Immunoglobulin production is further increased in lupus patient B cells by co-culturing with lupus patient NKT cells where IgG, IgM and IgA production increased about 3-10 fold, 2-4 fold and 2-4 fold, respectively. Moreover, lupus patient NKT cells help lupus patient B cells secrete anti-dsDNA IgG autoantibody, a hallmark of the disease. The addition of anti-human CD1d antibody inhibits production of antibodies by normal and lupus patient B cells. Of particular significance is the discovery that the addition of anti-CD1d antibody significantly reduces anti-dsDNA IgG production. (Figure 1, attached) These data suggest that NKT cells augment antibody production and isotype switching through the recognition of CD1d on B cells.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that making willful false statements and the like are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the applications or any patent issuing thereon.

Dated: October 29, 2007

Respectfully submitted,



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SUCCESSFUL TREATMENT OF AUTOIMMUNITY IN NZB/NZW F₁ MICE WITH MONOCLONAL ANTIBODY TO L3T4

BY DAVID WOFSY AND WILLIAM E. SEAMAN

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The advent of hybridoma technology has rekindled hopes of using antibodies as specific therapeutic agents (1-4). Several reports have focused attention on the potential use of monoclonal antibodies (mAb)¹ either as antitumor agents (4-15) or as immunosuppressive agents designed to facilitate organ transplantation (16-21). Early therapeutic trials in humans have demonstrated that certain mAb can be used to retard the progression of lymphoid malignancy (10, 11) or to reverse renal allograft rejection (17, 18). However, in most cases, the beneficial effects of treatment have been transient (4, 12-17) due in part to the development of host immunity to the administered mAb (4, 9, 16, 17).

Recently, (22) we treated three murine models for systemic lupus erythematosus (SLE) with biweekly injections of rat anti-T cell (anti-Thy-1.2) mAb in order to examine the role of T cells in the pathogenesis of murine lupus and to explore the possibility of using antilymphocyte mAb to treat autoimmunity. Treatment with anti-Thy-1.2 substantially reduced circulating T cells in all three strains (MRL/lpr, NZB/NZW F₁, BXSB), despite development of antibodies to rat Ig. Therapy was beneficial in MRL/lpr mice. It reduced autoantibody production, retarded renal disease, and markedly prolonged life. In contrast, treatment did not reduce autoimmunity in NZB/NZW F₁ (B/W) mice, and it caused fatal anaphylaxis in BXSB mice. These findings demonstrate that antilymphocyte mAb can serve as specific probes to examine the cells that contribute to autoimmunity. The results in MRL/lpr mice illustrate the potential therapeutic value of antilymphocyte mAb when a pathogenic cell subset can be identified. However, the results in BXSB mice emphasize equally the potential hazards of the host immune response to treatment with mAb.

The development of a new rat mAb (GK1.5) reactive with mouse helper T cells provides an opportunity for a more selective approach to the study of T cell regulation of autoimmunity in murine lupus. This antibody recognizes a glycoprotein antigen, designated L3T4, that is the mouse homologue for the human T cell antigen termed Leu-3 or T4 (23). Like Leu-3/T4 in humans, L3T4

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¹Abbreviations used in this paper: BGG, bovine gamma globulin; BSA, bovine serum albumin; BUN, blood urea nitrogen; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; MHA II, class II major histocompatibility antigens; SLE, systemic lupus erythematosus; TLI, total lymphoid irradiation.

participates in the T cell response to class II major histocompatibility antigens (MHA II) on antigen-presenting cells (24–28). Antibody to L3T4 inhibits most MHA II-dependent T cell responses in vitro (25–27). Therefore, administration of mAb against L3T4 might alter immune responses in vivo and thereby influence the course of autoimmune disease. To test this hypothesis, we treated B/W female mice with weekly injections of GK1.5 (anti-L3T4) beginning at age 4 mo. Treatment produced a sustained reduction in circulating T cells expressing L3T4. It also dramatically reduced autoantibody concentrations, retarded renal disease, and prolonged life. Mice treated with rat anti-L3T4 mAb developed little or no antibody to the administered rat Ig. In contrast, control mice treated with purified nonimmune rat IgG developed high titers of antibody to rat Ig. These findings establish that autoimmune disease in B/W mice is T cell-dependent. They further demonstrate that: (a) mAb directed against helper T cells can significantly improve autoimmune disease in B/W mice; and (b) the beneficial effects of treatment with anti-L3T4 may not be complicated by the development of a host immune response to therapy.

Materials and Methods

Mice. B/W mice were bred in our colony at the San Francisco Veterans Administration Medical Center from NZB females, purchased from The Jackson Laboratory, Bar Harbor, ME, and NZW males purchased from Simonsen Laboratory, Gilroy, CA.

Treatment Regimen. 10 mice were treated with a rat IgG2b mAb against L3T4, designated GK1.5 (23). The hybridoma used to produce GK1.5 was generously provided by Dr. Frank W. Fitch, University of Chicago. Antibody to L3T4 was harvested as ascites from sublethally irradiated BALB/c mice, partially purified by ammonium sulfate precipitation, dialyzed against phosphate-buffered saline, and quantified by protein electrophoresis and measurement of optical density. Treatment consisted of an initial intravenous injection of 2 mg of anti-L3T4, followed by weekly intraperitoneal injections (2 mg per mouse). In preliminary studies, we determined that an intravenous injection of 2 mg of anti-L3T4 produces saturation binding of circulating and splenic target cells, and substantial, though incomplete, binding of L3T4⁺ lymph node cells and thymocytes (unpublished data). Two control groups were also studied: one group (10 mice) received weekly 2-mg injections of chromatographically purified rat IgG (Cappel Laboratories, Cochranville, PA); the other group (11 mice) received saline.

Fluorescence Analysis of Lymphocyte Subpopulations. Peripheral blood was obtained from individual mice by retroorbital bleeding into heparinized pipettes. Mononuclear cells were separated by centrifugation over Lympholyte-M (Cedarlane Laboratories, Ontario, Canada), and quantified using a Coulter ZBI cell counter (Coulter Electronics, Inc., Hialeah, FL). Mononuclear cell subsets were identified by staining with fluorescein-conjugated mAb, as described previously (29). The antibodies used for analysis included: anti-Thy-1.2 (30-H12) to identify all T cells, anti-L3T4 (GK1.5) and anti-Lyt-2 (53-6) to identify the major T cell subsets, and anti-ThB (53-9) to identify B cells. Fluorescein-conjugated mouse anti-rat kappa chain mAb (MAR 18.5; B-D FACS Systems, Sunnyvale, CA) was used to detect anti-L3T4 on target cell surfaces. Except as noted, fluorescence analysis was always performed immediately before treatment, i.e., 1 wk after the previous injection.

Assessment of Renal Disease. Blood urea nitrogen (BUN) was determined on samples from individual mice using a CentrifChem System 400 (Union Carbide, Danbury, CT). Proteinuria was measured colorimetrically by the use of Albustix (Miles Laboratories Inc., Elkhart, IN). This produces an approximation of proteinuria as follows: trace, 10 mg/dl; 1+, 30 mg/dl; 2+, 100 mg/dl; 3+, 300 mg/dl; 4+, 1,000 mg/dl. All measurements of proteinuria were performed by an observer who had no knowledge of the given treatment.

Measurement of Serum Antibodies to Double-stranded DNA. Anti-DNA antibody concentrations were assessed by a solid phase enzyme-linked immunosorbent assay (ELISA)

derived from the method of Fish and Ziff (30). Immulon II polyvinyl microtitration trays were coated sequentially with a 50 $\mu\text{g/ml}$ solution of poly-L-lysine (Sigma Chemical Co., St. Louis, MO) in 0.1 M Tris-HCl buffer, pH 7.3 (75 $\mu\text{l/well}$), a 20 $\mu\text{g/ml}$ solution of poly(dA-dT) (Sigma Chemical Co.) in Tris buffer (75 $\mu\text{l/well}$), and a 2% solution of bovine serum albumin (BSA) (Sigma Chemical Co.) in Tris buffer (250 $\mu\text{l/well}$). Serum samples that had been serially diluted in Tris buffer containing 1% BSA and 2% bovine gamma globulin (BGG) (Sigma Chemical Co.) were then incubated at 75 $\mu\text{l/well}$ for 1.5 h at room temperature. The wells were then washed five times and incubated with peroxidase-conjugated goat anti-mouse Ig that had been extensively absorbed with rat Ig (0.3 $\mu\text{g/well}$; Cappel Laboratories). After incubation at room temperature for 1 h, the wells were washed five times and treated in the dark for 10 min with *o*-phenylenediamine (100 $\mu\text{l/well}$). The enzymatic reaction was stopped with 2 N H₂SO₄ (200 $\mu\text{l/well}$), and absorbance was measured at 490 nm. Serum from adult MRL/lpr and C57BL/6 mice served as positive and negative controls, respectively. The specificity of the assay was demonstrated using wells that had not been coated with poly(dA-dT).

Measurement of Serum Antibodies to Rat Ig. Antibodies to rat Ig were measured by an ELISA technique similar to the one described above. Briefly, Immulon II polyvinyl microtitration trays were coated with rat Ig mAb (0.5 μg 30-H12/well) followed by 2% BSA in 0.1 M Tris-HCl buffer. Serum samples were serially diluted in Tris buffer containing 1% BSA and 2% BGG, then incubated at 75 $\mu\text{l/well}$ for 1.5 h at room temperature. The wells were then washed, incubated with peroxidase-conjugated goat anti-mouse Ig that had been absorbed with rat Ig, washed again, and then treated in the dark with *o*-phenylenediamine exactly as described above. Mouse anti-rat kappa chain mAb (MAR 18.5) and normal mouse serum served as positive and negative controls, respectively.

Measurement of Serum IgM and IgG Levels. Serum concentrations of mouse IgM and IgG were measured by ELISA. Immulon II polyvinyl microtitration trays were coated with a 10 $\mu\text{g/ml}$ solution of either goat anti-mouse IgG or goat anti-mouse IgM (both, Cappel Laboratories), followed by 2% BSA in 0.1 M Tris-HCl buffer. Serum samples were serially diluted in Tris buffer containing 1% BSA and 2% BGG, then incubated at 75 $\mu\text{l/well}$ for 1.5 h at room temperature. The wells were then washed, incubated with peroxidase-conjugated goat anti-mouse Ig that had been absorbed with rat Ig, washed again, and then treated in the dark with *o*-phenylenediamine, as described above. Purified mouse IgM and IgG (Miles Laboratories) were used to generate standard curves and document the specificity of the assay.

Results

Effect of Age on Circulating Lymphocyte Subpopulations in Untreated B/W Mice. In control B/W mice treated with saline, there was a spontaneous age-dependent reduction in circulating T lymphocytes (Fig. 1, A and B). The number of circulating B lymphocytes remained relatively constant (Fig. 1C). Although T cell counts fell by ~50% between 4 and 8 mo of age, L3T4⁺ cells and Lyt-2⁺ cells were comparably affected and, therefore, the L3T4/Lyt-2 (helper/suppressor) ratio remained constant (Fig. 1D). Treatment with weekly injections of nonimmune rat IgG did not affect the spontaneous progressive changes in circulating lymphocyte subpopulations in B/W mice.

Effect of Treatment With Anti-L3T4 mAb on Circulating Lymphocyte Subpopulations. Treatment of B/W mice with weekly injections of anti-L3T4 mAb produced a dramatic and sustained reduction in circulating L3T4⁺ lymphocytes (Fig. 1A). Circulating L3T4⁺ cells were reduced by 30% 24 h after the first injection, by 90% 1 wk later, and by 95% thereafter. Fluorescence analysis of circulating lymphocytes using mouse anti-rat kappa chain mAb demonstrated persistence of administered antibody on the few remaining target cells 7 d after

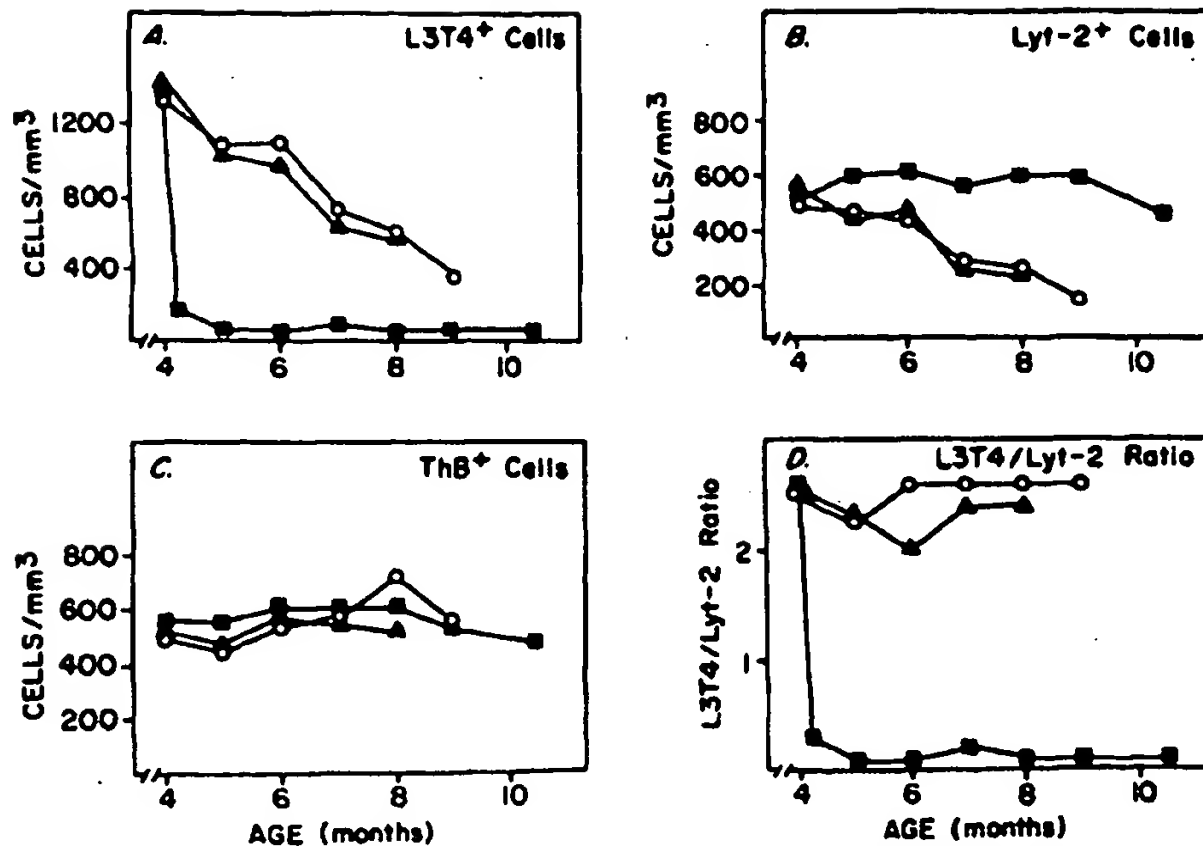


FIGURE 1. Circulating lymphocyte subpopulations in B/W mice treated with weekly injections of saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■) beginning at age 4 mo. Helper T cells, suppressor/cytotoxic T cells, and B cells were identified using fluorescein-conjugated anti-L3T4 mAb (A), anti-Lyt-2 (B), and anti-Thy B (C), respectively. The relative proportion of L3T4⁺ and Lyt-2⁺ T cells is shown in D.

injection. Staining with fluorescein-conjugated anti-Thy-1.2 mAb and anti-Lyt-2 established that, after 1 wk of therapy, >90% of circulating T cells in treated mice expressed Lyt-2, indicating that the reduction in L3T4⁺ T cells reflected target cell depletion rather than antigen modulation. The effect of treatment with anti-L3T4 was specific. The number of circulating B cells and Lyt-2⁺ T cells remained stable in treated mice (Fig. 1, B and C). The mean L3T4/Lyt-2 ratio in treated mice was 0.1:1, compared with 2.6:1, in untreated mice.

Host Immune Response to Treatment. Control mice treated with nonimmune rat IgG all developed high titers of antibody to rat Ig (Fig. 2). Host antibodies were detectable 7 d after the first injection of rat IgG and rapidly rose to a peak mean titer of 1:4,500. In contrast, only 3 of 10 mice treated with rat anti-L3T4 mAb developed antibodies to rat Ig. In these mice, antibodies to rat Ig first appeared after 2–3 mo of therapy and were present only in relatively low titer (<1:400). 7 of the 10 treated mice never developed detectable antibody to rat Ig. This is in striking contrast to our previous observation that B/W mice treated with rat anti-Thy-1.2 mAb of the same isotype as anti-L3T4 all develop high titers of anti-rat Ig (22).

Anti-L3T4 Reduces Autoimmunity. Treatment of B/W mice with anti-L3T4 mAb prevented development of high titers of antibody to double-stranded DNA (Fig. 3). Although small amounts of anti-DNA antibody appeared within the first

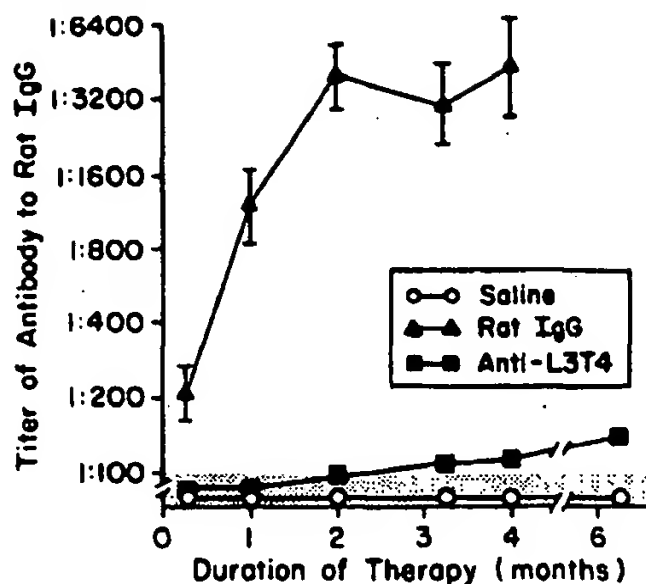


FIGURE 2. Geometric mean titer (\bar{x} SEM) of antibodies to rat Ig in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■). The shaded area indicates assay results using normal mouse sera as negative controls.

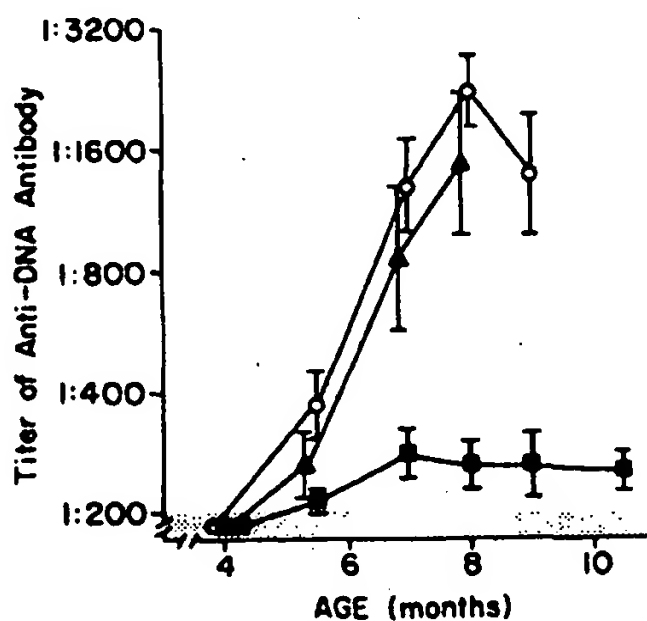


FIGURE 3. Geometric mean titer (\bar{x} SEM) of antibody to double-stranded DNA in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■). The shaded area indicates the titer of anti-DNA antibody (<1:200) in normal (C57BL/6) mice.

2 mo of therapy with anti-L3T4, the titers of anti-DNA antibody were markedly lower in treated mice than in control mice, and the titers remained low throughout the duration of therapy. At age 8 mo, the geometric mean titer of anti-DNA antibody was 1:2,200 \bar{x} 1.2 in mice treated with saline, 1:1,500 \bar{x} 1.5 in mice treated with nonimmune rat Ig, and 1:270 \bar{x} 1.1 in mice treated with anti-L3T4 ($P < 0.0005$ compared with either control group; Student's *t* test). The reduction in anti-DNA antibody did not reflect a generalized reduction in total Ig levels in treated mice (Table I). After 4 mo of treatment, there was a slight reduction in the mean IgG concentration in treated mice that was not statistically significant.

TABLE I
Effect of Treatment on Ig Concentration

Treatment	IgM*		IgG*	
	4-mo-old	8-mo-old	4-mo-old	8-mo-old
Saline	9.5 ± 0.2	9.2 ± 0.2	15.5 ± 0.2	15.7 ± 0.2
Rat IgG	9.6 ± 0.2	9.6 ± 0.2	15.7 ± 0.3	15.5 ± 0.6
Anti-L3T4	9.6 ± 0.2	10.3 ± 0.2 [‡]	15.5 ± 0.3	15.2 ± 0.3

* Log₂ titer ($\bar{x} \pm$ SEM) as measured by ELISA.

[‡] $P < 0.05$ compared to pretreatment IgM levels and to IgM levels in both control groups.

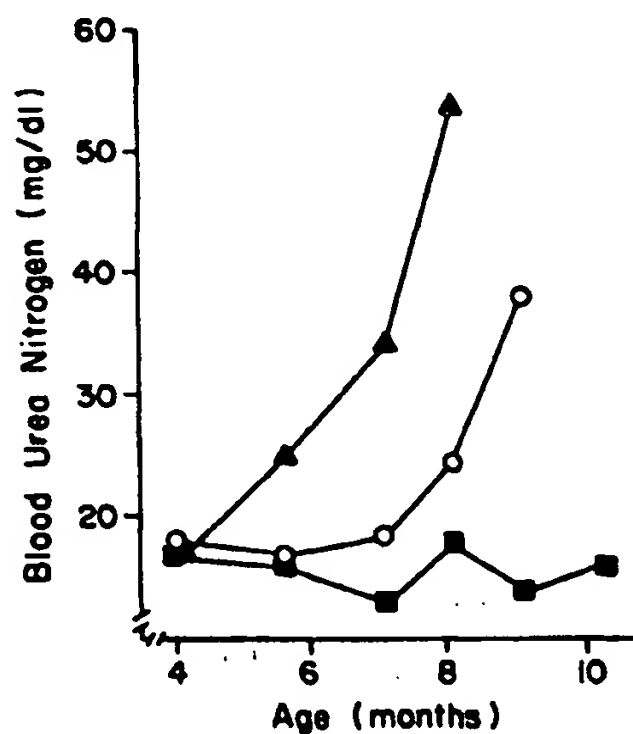


FIGURE 4. Mean BUN in B/W mice treated with saline (O), nonimmune rat IgG (Δ) or anti-L3T4 mAb (■).

However, there was a significant increase in the mean IgM concentration in treated mice compared with pretreatment levels, and with IgM levels in control mice. Increased plasma IgM has been reported previously (31) in association with successful treatment of B/W mice.

Anti-L3T4 Reduces Renal Disease. The reduction in anti-DNA antibody concentration in mice treated with anti-L3T4 was associated with a significant reduction in renal disease. Renal function, as assessed by BUN, deteriorated steadily in both control groups, but was preserved in mice treated with anti-L3T4 (Fig. 4). Renal insufficiency occurred earliest in mice treated with non-immune rat IgG, perhaps as a consequence of the host immune response to rat Ig. At age 7 mo, the mean BUN in mice treated with rat IgG was 34.2 ± 12.1 mg/dl, compared to 18.5 ± 2.7 mg/dl in mice treated with saline ($P < 0.05$; Mann-Whitney U test) and 13.1 ± 0.8 mg/dl in mice treated with anti-L3T4 ($P < 0.01$ compared with rat IgG treatment; $P < 0.05$ compared with saline treatment). By 9 mo, the mean BUN in mice treated with saline rose to 38.0 ± 11.6 mg/dl, compared with 14.3 ± 0.9 mg/dl in mice treated with anti-L3T4 (P

< 0.01). Severe proteinuria ($\geq 3+$) developed in 50–60% of mice in each control group, but in none of the mice treated with anti-L3T4 ($P < 0.05$; chi-square analysis) (Fig. 5). Moderate proteinuria ($2+$) developed in 16 of 21 control mice but in only 2 of 10 treated mice ($P < 0.05$). These two mice were among the three treated mice that developed low titers of antibody to rat Ig, suggesting that the immune response to rat Ig may have contributed to the development of proteinuria.

Anti-L3T4 Prolongs Survival. Treatment with anti-L3T4 mAb dramatically prolonged life (Fig. 6). Median survival was 9.3 mo in mice treated with saline and 7.6 mo in mice treated with rat IgG. In contrast, all of the mice treated with anti-L3T4 are still alive at 10 mo. The difference in 10-mo survival between

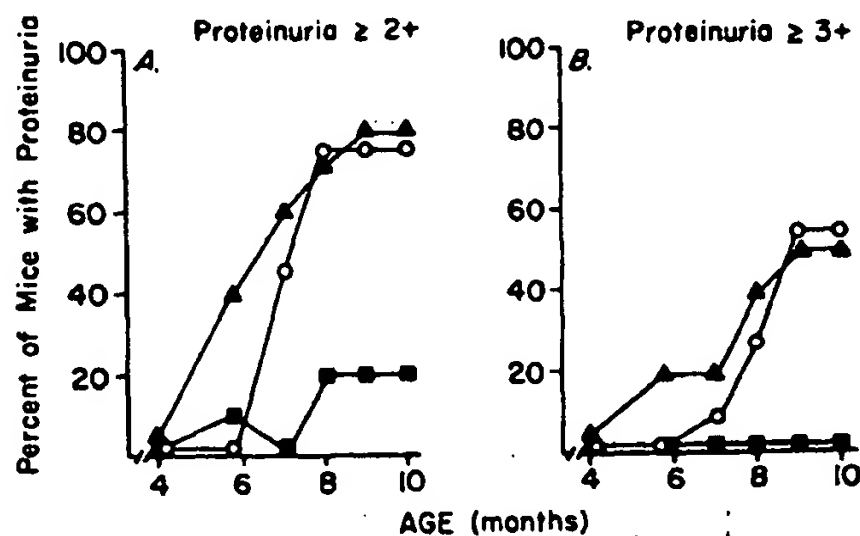


FIGURE 5. Frequency of significant proteinuria in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■). (A) Percent of mice with proteinuria $\geq 2+$ (100 mg/dl). (B) Percent of mice with proteinuria $\geq 3+$ (300 mg/dl). To reflect accurately the development of renal disease in all mice, each point reflects the current level of proteinuria in surviving mice, as well as the last measurement of proteinuria in deceased mice.

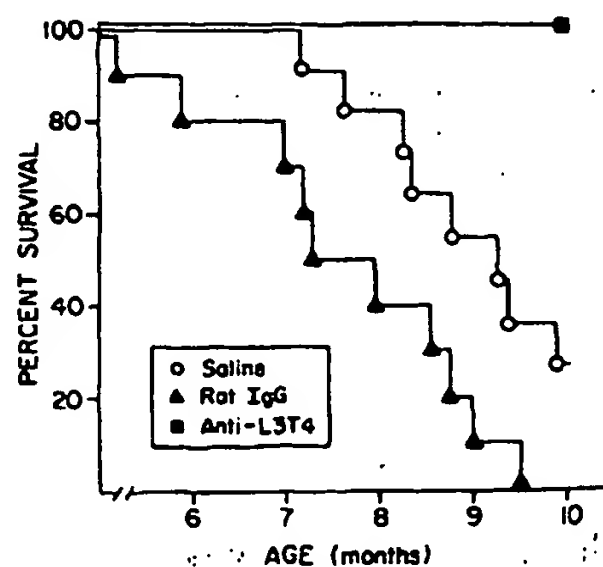


FIGURE 6. Survival in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■).

mice treated with anti-L3T4 and control mice is strongly significant ($P < 0.001$ compared with the saline control group; $P < 0.00001$ compared with the rat IgG control group; chi-square analysis). The difference in mean survival between the two control groups is also statistically significant ($P < 0.05$; t test), i.e., treatment with nonimmune rat Ig accelerated mortality.

Discussion

To clarify the role of helper T cells in the pathogenesis of autoimmune disease in the B/W model for SLE, we treated female B/W mice with repeated injections of a rat mAb directed against the mouse helper T cell antigen, L3T4. Treatment depleted circulating target cells, reduced autoantibody production, retarded renal disease, and prolonged life. Moreover, treated mice produced little or no host antibodies to the administered rat mAb. These findings provide the first clear demonstration that autoimmune disease in B/W mice is T cell dependent. They further suggest that mAb directed against specific helper T cell antigens may be effective in the treatment of certain autoimmune diseases, and that the therapeutic benefit may be achieved without provoking an undesirable, and potentially hazardous, immune response to the mAb.

For two decades, the B/W strain has been studied (32, 33) as a model for SLE in humans. Early studies (34–37) focused attention on T cell abnormalities in B/W mice, emphasizing defects in suppressor T cell number and function. Recent studies, however, have cast doubt on the significance of these T cell abnormalities. For example, neonatal thymectomy has little effect on the course of autoimmunity in B/W mice (38), but elimination of a specific subset of B cells prevents autoimmune disease (39). Other studies, demonstrating B cell abnormalities, and indicating that B cell abnormalities may precede demonstrable T cell abnormalities in B/W mice, have suggested that autoimmunity in these mice may reflect a primary B cell defect, and that T cell defects may be secondary phenomena (40–43). Our results demonstrate conclusively that T cells, specifically helper T cells, play an important role in the pathogenesis of autoimmune disease in B/W mice. If primary defects occur in other cell types, particularly B lymphocytes, these cells nonetheless require helper T cells for the expression of disease.

It has been postulated that autoimmunity in B/W mice might reflect selective loss of suppressor T cells (36, 37), perhaps as a consequence of the action of natural thymocytotoxic antibodies (44, 45). Our findings in untreated B/W mice show that, although progressive T lymphocytopenia occurs, there is no selective effect on suppressor (Lyt-2⁺) T cells. Rather, both major T cell subsets are comparably reduced, and the L3T4/Lyt-2 ratio is unchanged. There is, thus, no numerical imbalance between helper and suppressor/cytotoxic T cells, although functional differences cannot be excluded.

The L3T4 molecule is expressed on a distinct subpopulation of T cells previously referred to descriptively as helper T cells. More precisely, L3T4 identifies T cells that respond to MHA II on antigen-presenting cells (24–27). The functional importance of the L3T4 molecule is demonstrated by the observation that mAb against L3T4 blocks in vitro T cell responses to MHA II (25–27). The beneficial effects of anti-L3T4 in B/W mice may, therefore, be due not only to target cell depletion, but also to direct inhibition of T cell responses that

are dependent on the recognition of MHA II. The latter mechanism would imply that autoimmunity, like immunity to foreign antigens, requires a T cell response to MHA II. Our results are compatible with this possibility, but they do not prove it. We previously (22) treated B/W mice with mAb against Thy-1.2, an antigen expressed on all T cells. Although treatment reduced circulating L3T4⁺ T cells by 75%, there was a comparable reduction in Lyt-2⁺ T cells, and no improvement in autoimmunity. The failure of anti-Thy-1.2 to improve autoimmunity despite depletion of L3T4⁺ cells suggests either that: (a) regulation of autoimmunity in B/W mice involves a balance between L3T4⁺ cells and Lyt-2⁺ cells that is not disturbed by treatment with anti-Thy-1.2; or (b) successful treatment with anti-L3T4 requires functional impairment of the L3T4 molecule.

An important observation in our present studies was the relative lack of host immunity to the anti-L3T4 antibody. Previous attempts to use mAb as therapeutic agents in humans and in mice have been complicated by the development of a host immune response to the mAb (4, 9, 16, 17, 22). In B/W mice treated with rat anti-Thy-1.2 mAb (subclass IgG2b), the development of antibody to rat Ig was associated with accelerated mortality, even though autoimmunity was not affected (22). In autoimmune BXSB mice, treatment with anti-Thy-1.2 caused fatal anaphylaxis (22). The current study reemphasizes the potential hazards of treatment with foreign Ig, because administration of purified nonimmune rat IgG significantly reduced survival. In this context, the weak host immune response to rat anti-L3T4 mAb (subclass IgG2b) takes on added significance. The ability to administer anti-L3T4 without generating high titers of host antibody to rat Ig may not only maximize the therapeutic effect of anti-L3T4, it may also minimize the risks associated with the host immune response to foreign protein.

The lack of host immunity to repeated injections of anti-L3T4 is the first demonstration that anti-L3T4 can interrupt the immune response *in vivo*. It remains to be determined if anti-L3T4 will block the *in vivo* response to foreign antigens other than itself, although it has been demonstrated (25) that anti-L3T4 can block *in vitro* responses to foreign antigens that are presented in association with MHA II. Immune recognition of MHA II can also be blocked both *in vitro* and *in vivo* by mAb against MHA II (46–49). One group of researchers (50–52) have treated murine models for several autoimmune diseases with mAb against MHA II. This treatment was effective in experimental autoimmune encephalitis (50) and experimentally induced myasthenia gravis (51). It also increased survival in B/W mice, even though it did not reduce autoantibody production (52). In mice, MHA II are expressed on macrophages, B cells, and activated T cells (53), but it is not known whether successful treatment with mAb against MHA II is associated with depletion of these cell types. In humans, MHA II are expressed not only on certain lymphoid cells, but also on blood vessel endothelial cells (54). This may account for the severe toxicity of mAb against MHA II in monkeys (55), which may complicate the use of anti-MHA II in humans. Unlike MHA II, L3T4 appears to be restricted in its expression to a distinct subpopulation of T lymphocytes and T lymphocyte precursors (24). Therefore, anti-L3T4 mAb may provide an alternative to the use of anti-MHA II mAb that would be more selective in its effect on the immune system. The human homologue for the

L3T4 antigen has been identified (23), and mAb against this antigen have already been used to prolong renal allograft survival in nonhuman primates (19).

Treatment of B/W mice with anti-L3T4 had profound effects on the immune system that raise questions about the immune competence of treated mice. The reduction in L3T4⁺ T cells creates an imbalance between T cell subpopulations that resembles the distribution of T cell subsets in acquired immunodeficiency syndrome (AIDS) in people (56). The beneficial reduction in antibodies to double-stranded DNA and antibodies to rat Ig may reflect a more generalized impairment of humoral immunity. Our studies do not establish the significance of these alterations in cellular and humoral immunity, but they do provide some preliminary insight. Despite a 10-fold reduction in autoantibody concentration, treatment with anti-L3T4 did not significantly depress Ig levels. Moreover, treated mice did not develop infectious complications, even though they were housed without special precautions in our main animal colony. Studies are currently in progress in our laboratory to determine the precise effects of treatment with anti-L3T4 on immune competence in normal and autoimmune mice.

The immunologic abnormalities in B/W mice closely parallel the immunologic abnormalities underlying SLE in humans. T lymphocytopenia, impaired suppressor T cell function, reduced lymphokine production, and B cell hyperactivity all occur in humans with SLE as well as in B/W mice, but the significance of these abnormalities remains controversial (32, 57-60). There is no fixed abnormality in the relative proportion of circulating T cell subsets in either B/W mice or humans with SLE (61), but our results make it clear that this does not exclude the possibility that T cells regulate autoimmunity in SLE. The similarities between SLE in humans and SLE in B/W mice suggest that they may share common pathogenetic mechanisms. Our findings in B/W mice therefore support the hypothesis that autoimmunity in people with SLE is regulated by T cells. This hypothesis is consistent with recent studies indicating that total lymphoid irradiation (TLI) improves lupus nephritis in humans (62-64). Although the effect of TLI on lymphocyte subsets in SLE has not yet been determined, TLI causes prolonged depletion and impaired function of Leu-3/T4⁺ cells in people with rheumatoid arthritis (65-67). These observations add weight to the possibility that treatment with mAb against Leu-3/T4 would favorably influence the course of autoimmune disease in people with SLE. However, we believe that such treatment would be premature until the effects of anti-L3T4 on normal immune function have been thoroughly investigated.

Summary

Autoimmune NZB/NZW mice were treated with weekly injections of monoclonal antibody (mAb) to L3T4, an antigen expressed on a distinct subpopulation of T cells that respond to class II major histocompatibility antigens. Treatment with anti-L3T4 depleted circulating target cells, reduced autoantibody production, retarded renal disease, and prolonged life relative to control mice treated either with saline or with purified nonimmune rat IgG. These findings establish that autoimmune disease in NZB/NZW mice is regulated by T cells. In contrast to mice treated with nonimmune rat IgG, mice treated with rat anti-L3T4 mAb

developed little or no antibody to rat Ig. Thus, the benefits of treatment with anti-L3T4 were achieved while minimizing the risks associated with a host immune response to therapy. This study raises the possibility that treatment with mAb against Leu-3/T4, the human homologue for L3T4, might be effective in the treatment of certain autoimmune diseases in people.

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SUCCESSFUL TREATMENT OF AUTOIMMUNITY IN NZB/NZW F₁ MICE WITH MONOCLONAL ANTIBODY TO L3T4

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The advent of hybridoma technology has rekindled hopes of using antibodies as specific therapeutic agents (1-4). Several reports have focused attention on the potential use of monoclonal antibodies (mAb)¹ either as antitumor agents (4-15) or as immunosuppressive agents designed to facilitate organ transplantation (16-21). Early therapeutic trials in humans have demonstrated that certain mAb can be used to retard the progression of lymphoid malignancy (10, 11) or to reverse renal allograft rejection (17, 18). However, in most cases, the beneficial effects of treatment have been transient (4, 12-17) due in part to the development of host immunity to the administered mAb (4, 9, 16, 17).

Recently, (22) we treated three murine models for systemic lupus erythematosus (SLE) with biweekly injections of rat anti-T cell (anti-Thy-1.2) mAb in order to examine the role of T cells in the pathogenesis of murine lupus and to explore the possibility of using antilymphocyte mAb to treat autoimmunity. Treatment with anti-Thy-1.2 substantially reduced circulating T cells in all three strains (MRL/lpr, NZB/NZW F₁, BXSB), despite development of antibodies to rat Ig. Therapy was beneficial in MRL/lpr mice. It reduced autoantibody production, retarded renal disease, and markedly prolonged life. In contrast, treatment did not reduce autoimmunity in NZB/NZW F₁ (B/W) mice, and it caused fatal anaphylaxis in BXSB mice. These findings demonstrate that antilymphocyte mAb can serve as specific probes to examine the cells that contribute to autoimmunity. The results in MRL/lpr mice illustrate the potential therapeutic value of antilymphocyte mAb when a pathogenic cell subset can be identified. However, the results in BXSB mice emphasize equally the potential hazards of the host immune response to treatment with mAb.

The development of a new rat mAb (GK1.5) reactive with mouse helper T cells provides an opportunity for a more selective approach to the study of T cell regulation of autoimmunity in murine lupus. This antibody recognizes a glycoprotein antigen, designated L3T4, that is the mouse homologue for the human T cell antigen termed Leu-3 or T4 (23). Like Leu-3/T4 in humans, L3T4

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¹Abbreviations used in this paper: BGG, bovine gamma globulin; BSA, bovine serum albumin; BUN, blood urea nitrogen; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; MHA II, class II major histocompatibility antigens; SLE, systemic lupus erythematosus; TLI, total lymphoid irradiation.

participates in the T cell response to class II major histocompatibility antigens (MHA II) on antigen-presenting cells (24-28). Antibody to L3T4 inhibits most MHA II-dependent T cell responses in vitro (25-27). Therefore, administration of mAb against L3T4 might alter immune responses in vivo and thereby influence the course of autoimmune disease. To test this hypothesis, we treated B/W female mice with weekly injections of GK1.5 (anti-L3T4) beginning at age 4 mo. Treatment produced a sustained reduction in circulating T cells expressing L3T4. It also dramatically reduced autoantibody concentrations, retarded renal disease, and prolonged life. Mice treated with rat anti-L3T4 mAb developed little or no antibody to the administered rat Ig. In contrast, control mice treated with purified nonimmune rat IgG developed high titers of antibody to rat Ig. These findings establish that autoimmune disease in B/W mice is T cell-dependent. They further demonstrate that: (a) mAb directed against helper T cells can significantly improve autoimmune disease in B/W mice; and (b) the beneficial effects of treatment with anti-L3T4 may not be complicated by the development of a host immune response to therapy.

Materials and Methods

Mice. B/W mice were bred in our colony at the San Francisco Veterans Administration Medical Center from NZB females, purchased from The Jackson Laboratory, Bar Harbor, ME, and NZW males purchased from Simonsen Laboratory, Gilroy, CA.

Treatment Regimen. 10 mice were treated with a rat IgG2b mAb against L3T4, designated GK1.5 (23). The hybridoma used to produce GK1.5 was generously provided by Dr. Frank W. Fitch, University of Chicago. Antibody to L3T4 was harvested as ascites from sublethally irradiated BALB/c mice, partially purified by ammonium sulfate precipitation, dialyzed against phosphate-buffered saline, and quantified by protein electrophoresis and measurement of optical density. Treatment consisted of an initial intravenous injection of 2 mg of anti-L3T4, followed by weekly intraperitoneal injections (2 mg per mouse). In preliminary studies, we determined that an intravenous injection of 2 mg of anti-L3T4 produces saturation binding of circulating and splenic target cells, and substantial, though incomplete, binding of L3T4⁺ lymph node cells and thymocytes (unpublished data). Two control groups were also studied: one group (10 mice) received weekly 2-mg injections of chromatographically purified rat IgG (Cappel Laboratories, Cochranville, PA); the other group (11 mice) received saline.

Fluorescence Analysis of Lymphocyte Subpopulations. Peripheral blood was obtained from individual mice by retroorbital bleeding into heparinized pipettes. Mononuclear cells were separated by centrifugation over Lympholyte-M (Cedarlane Laboratories, Ontario, Canada), and quantified using a Coulter ZBI cell counter (Coulter Electronics, Inc., Hialeah, FL). Mononuclear cell subsets were identified by staining with fluorescein-conjugated mAb, as described previously (29). The antibodies used for analysis included: anti-Thy-1.2 (30-H12) to identify all T cells, anti-L3T4 (GK1.5) and anti-Lyt-2 (53-6) to identify the major T cell subsets, and anti-ThB (53-9) to identify B cells. Fluorescein-conjugated mouse anti-rat kappa chain mAb (MAR 18.5; B-D FACS Systems, Sunnyvale, CA) was used to detect anti-L3T4 on target cell surfaces. Except as noted, fluorescence analysis was always performed immediately before treatment, i.e., 1 wk after the previous injection.

Assessment of Renal Disease. Blood urea nitrogen (BUN) was determined on samples from individual mice using a CentrifChem System 400 (Union Carbide, Danbury, CT). Proteinuria was measured colorimetrically by the use of Albustix (Miles Laboratories Inc., Elkhart, IN). This produces an approximation of proteinuria as follows: trace, 10 mg/dl; 1+, 30 mg/dl; 2+, 100 mg/dl; 3+, 300 mg/dl; 4+, 1,000 mg/dl. All measurements of proteinuria were performed by an observer who had no knowledge of the given treatment.

Measurement of Serum Antibodies to Double-stranded DNA. Anti-DNA antibody concentrations were assessed by a solid phase enzyme-linked immunosorbent assay (ELISA)

derived from the method of Fish and Ziff (30). Immulon II polyvinyl microtitration trays were coated sequentially with a 50 µg/ml solution of poly-L-lysine (Sigma Chemical Co., St. Louis, MO) in 0.1 M Tris-HCl buffer, pH 7.3 (75 µl/well), a 20 µg/ml solution of poly(dA-dT) (Sigma Chemical Co.) in Tris buffer (75 µl/well), and a 2% solution of bovine serum albumin (BSA) (Sigma Chemical Co.) in Tris buffer (250 µl/well). Serum samples that had been serially diluted in Tris buffer containing 1% BSA and 2% bovine gamma globulin (BGG) (Sigma Chemical Co.) were then incubated at 75 µl/well for 1.5 h at room temperature. The wells were then washed five times and incubated with peroxidase-conjugated goat anti-mouse Ig that had been extensively absorbed with rat Ig (0.3 µg/well; Cappel Laboratories). After incubation at room temperature for 1 h, the wells were washed five times and treated in the dark for 10 min with o-phenylenediamine (100 µl/well). The enzymatic reaction was stopped with 2 N H₂SO₄ (200 µl/well), and absorbance was measured at 490 nm. Serum from adult MRL/lpr and C57BL/6 mice served as positive and negative controls, respectively. The specificity of the assay was demonstrated using wells that had not been coated with poly(dA-dT).

Measurement of Serum Antibodies to Rat Ig. Antibodies to rat Ig were measured by an ELISA technique similar to the one described above. Briefly, Immulon II polyvinyl microtitration trays were coated with rat Ig mAb (0.5 µg 30-H12/well) followed by 2% BSA in 0.1 M Tris-HCl buffer. Serum samples were serially diluted in Tris buffer containing 1% BSA and 2% BGG, then incubated at 75 µl/well for 1.5 h at room temperature. The wells were then washed, incubated with peroxidase-conjugated goat anti-mouse Ig that had been absorbed with rat Ig, washed again, and then treated in the dark with o-phenylenediamine exactly as described above. Mouse anti-rat kappa chain mAb (MAR 18.5) and normal mouse serum served as positive and negative controls, respectively.

Measurement of Serum IgM and IgG Levels. Serum concentrations of mouse IgM and IgG were measured by ELISA. Immulon II polyvinyl microtitration trays were coated with a 10 µg/ml solution of either goat anti-mouse IgG or goat anti-mouse IgM (both, Cappel Laboratories), followed by 2% BSA in 0.1 M Tris-HCl buffer. Serum samples were serially diluted in Tris buffer containing 1% BSA and 2% BGG, then incubated at 75 µl/well for 1.5 h at room temperature. The wells were then washed, incubated with peroxidase-conjugated goat anti-mouse Ig that had been absorbed with rat Ig, washed again, and then treated in the dark with o-phenylenediamine, as described above. Purified mouse IgM and IgG (Miles Laboratories) were used to generate standard curves and document the specificity of the assay.

Results

Effect of Age on Circulating Lymphocyte Subpopulations in Untreated B/W Mice. In control B/W mice treated with saline, there was a spontaneous age-dependent reduction in circulating T lymphocytes (Fig. 1, A and B). The number of circulating B lymphocytes remained relatively constant (Fig. 1C). Although T cell counts fell by ~50% between 4 and 8 mo of age, L3T4⁺ cells and Lyt-2⁺ cells were comparably affected and, therefore, the L3T4/Lyt-2 (helper/suppressor) ratio remained constant (Fig. 1D). Treatment with weekly injections of nonimmune rat IgG did not affect the spontaneous progressive changes in circulating lymphocyte subpopulations in B/W mice.

Effect of Treatment With Anti-L3T4 mAb on Circulating Lymphocyte Subpopulations. Treatment of B/W mice with weekly injections of anti-L3T4 mAb produced a dramatic and sustained reduction in circulating L3T4⁺ lymphocytes (Fig. 1A). Circulating L3T4⁺ cells were reduced by 30% 24 h after the first injection, by 90% 1 wk later, and by 95% thereafter. Fluorescence analysis of circulating lymphocytes using mouse anti-rat kappa chain mAb demonstrated persistence of administered antibody on the few remaining target cells 7 d after

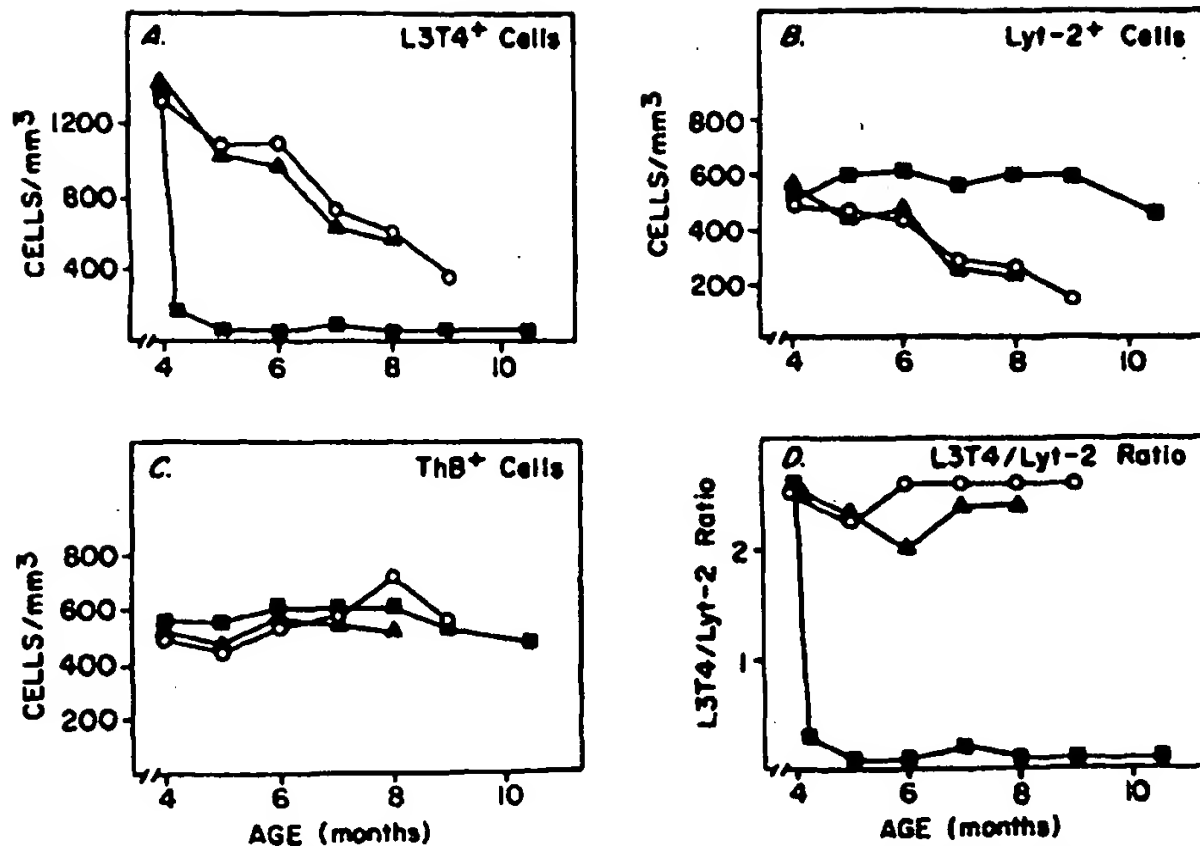


FIGURE 1. Circulating lymphocyte subpopulations in B/W mice treated with weekly injections of saline (O), nonimmune rat IgG (Δ), or anti-L3T4 mAb (■) beginning at age 4 mo. Helper T cells, suppressor/cytotoxic T cells, and B cells were identified using fluorescein-conjugated anti-L3T4 mAb (A), anti-Lyt-2 (B), and anti-Thy B (C), respectively. The relative proportion of L3T4⁺ and Lyt-2⁺ T cells is shown in D.

injection. Staining with fluorescein-conjugated anti-Thy-1.2 mAb and anti-Lyt-2 established that, after 1 wk of therapy, >90% of circulating T cells in treated mice expressed Lyt-2, indicating that the reduction in L3T4⁺ T cells reflected target cell depletion rather than antigen modulation. The effect of treatment with anti-L3T4 was specific. The number of circulating B cells and Lyt-2⁺ T cells remained stable in treated mice (Fig. 1, B and C). The mean L3T4/Lyt-2 ratio in treated mice was 0.1:1, compared with 2.6:1, in untreated mice.

Host Immune Response to Treatment. Control mice treated with nonimmune rat IgG all developed high titers of antibody to rat Ig (Fig. 2). Host antibodies were detectable 7 d after the first injection of rat IgG and rapidly rose to a peak mean titer of 1:4,500. In contrast, only 3 of 10 mice treated with rat anti-L3T4 mAb developed antibodies to rat Ig. In these mice, antibodies to rat Ig first appeared after 2–3 mo of therapy and were present only in relatively low titer (<1:400). 7 of the 10 treated mice never developed detectable antibody to rat Ig. This is in striking contrast to our previous observation that B/W mice treated with rat anti-Thy-1.2 mAb of the same isotype as anti-L3T4 all develop high titers of anti-rat Ig (22).

Anti-L3T4 Reduces Autoimmunity. Treatment of B/W mice with anti-L3T4 mAb prevented development of high titers of antibody to double-stranded DNA (Fig. 3). Although small amounts of anti-DNA antibody appeared within the first

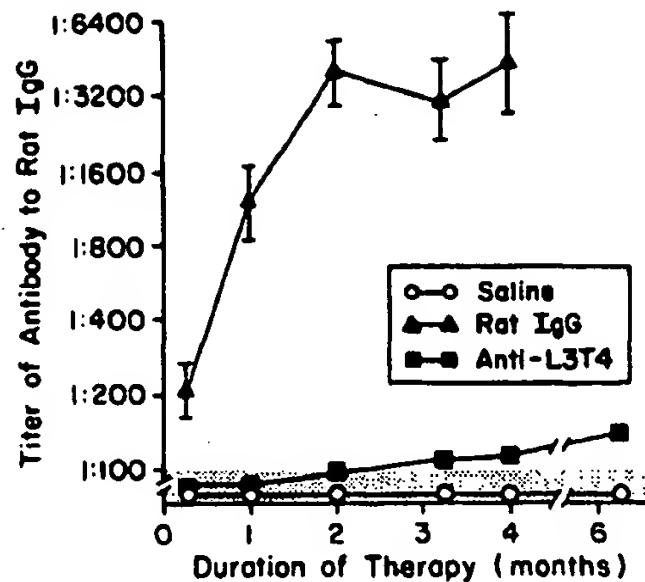


FIGURE 2. Geometric mean titer (\pm SEM) of antibodies to rat Ig in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■). The shaded area indicates assay results using normal mouse sera as negative controls.

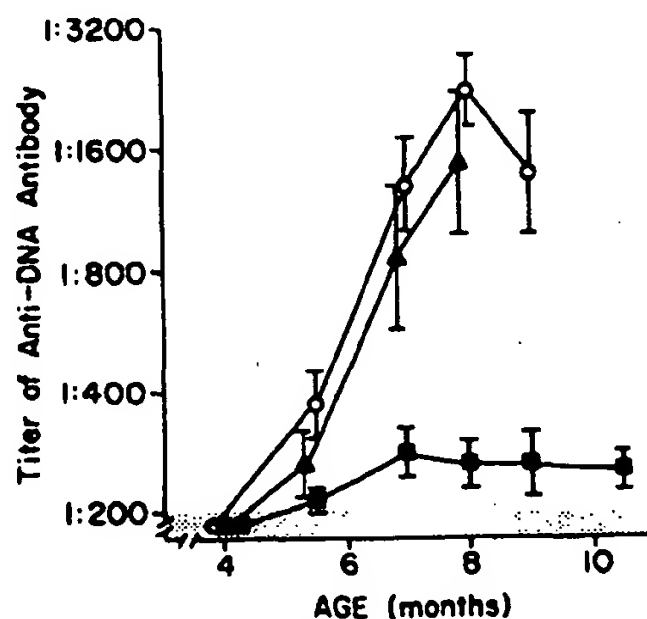


FIGURE 3. Geometric mean titer (\pm SEM) of antibody to double-stranded DNA in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■). The shaded area indicates the titer of anti-DNA antibody (<1:200) in normal (C57BL/6) mice.

2 mo of therapy with anti-L3T4, the titers of anti-DNA antibody were markedly lower in treated mice than in control mice, and the titers remained low throughout the duration of therapy. At age 8 mo, the geometric mean titer of anti-DNA antibody was $1:2,200 \pm 1.2$ in mice treated with saline, $1:1,500 \pm 1.5$ in mice treated with nonimmune rat Ig, and $1:270 \pm 1.1$ in mice treated with anti-L3T4 ($P < 0.0005$ compared with either control group; Student's *t* test). The reduction in anti-DNA antibody did not reflect a generalized reduction in total Ig levels in treated mice (Table I). After 4 mo of treatment, there was a slight reduction in the mean IgG concentration in treated mice that was not statistically significant.

TABLE I
Effect of Treatment on Ig Concentration

Treatment	IgM*		IgG*	
	4-mo-old	8-mo-old	4-mo-old	8-mo-old
Saline	9.5 ± 0.2	9.2 ± 0.2	15.5 ± 0.2	15.7 ± 0.2
Rat IgG	9.6 ± 0.2	9.6 ± 0.2	15.7 ± 0.3	15.5 ± 0.6
Anti-L3T4	9.6 ± 0.2	10.3 ± 0.2†	15.5 ± 0.3	15.2 ± 0.3

* Log₂ titer ($\bar{x} \pm$ SEM) as measured by ELISA.

† $P < 0.05$ compared to pretreatment IgM levels and to IgM levels in both control groups.

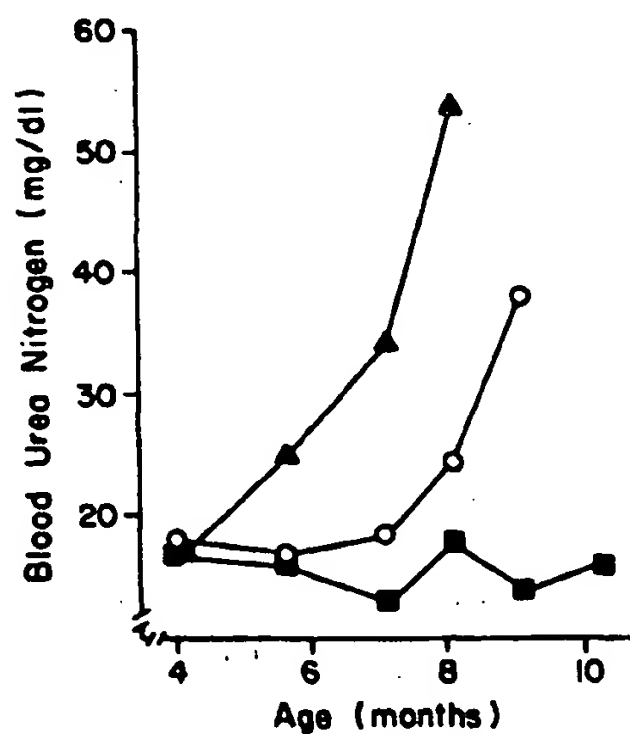


FIGURE 4. Mean BUN in B/W mice treated with saline (O), nonimmune rat IgG (Δ) or anti-L3T4 mAb (■).

However, there was a significant increase in the mean IgM concentration in treated mice compared with pretreatment levels, and with IgM levels in control mice. Increased plasma IgM has been reported previously (31) in association with successful treatment of B/W mice.

Anti-L3T4 Reduces Renal Disease. The reduction in anti-DNA antibody concentration in mice treated with anti-L3T4 was associated with a significant reduction in renal disease. Renal function, as assessed by BUN, deteriorated steadily in both control groups, but was preserved in mice treated with anti-L3T4 (Fig. 4). Renal insufficiency occurred earliest in mice treated with non-immune rat IgG, perhaps as a consequence of the host immune response to rat Ig. At age 7 mo, the mean BUN in mice treated with rat IgG was 34.2 ± 12.1 mg/dl, compared to 18.5 ± 2.7 mg/dl in mice treated with saline ($P < 0.05$; Mann-Whitney U test) and 13.1 ± 0.8 mg/dl in mice treated with anti-L3T4 ($P < 0.01$ compared with rat IgG treatment; $P < 0.05$ compared with saline treatment). By 9 mo, the mean BUN in mice treated with saline rose to 38.0 ± 11.6 mg/dl, compared with 14.3 ± 0.9 mg/dl in mice treated with anti-L3T4 (P

< 0.01). Severe proteinuria ($\geq 3+$) developed in 50–60% of mice in each control group, but in none of the mice treated with anti-L3T4 ($P < 0.05$; chi-square analysis) (Fig. 5). Moderate proteinuria ($2+$) developed in 16 of 21 control mice but in only 2 of 10 treated mice ($P < 0.05$). These two mice were among the three treated mice that developed low titers of antibody to rat Ig, suggesting that the immune response to rat Ig may have contributed to the development of proteinuria.

Anti-L3T4 Prolongs Survival. Treatment with anti-L3T4 mAb dramatically prolonged life (Fig. 6). Median survival was 9.3 mo in mice treated with saline and 7.6 mo in mice treated with rat IgG. In contrast, all of the mice treated with anti-L3T4 are still alive at 10 mo. The difference in 10-mo survival between

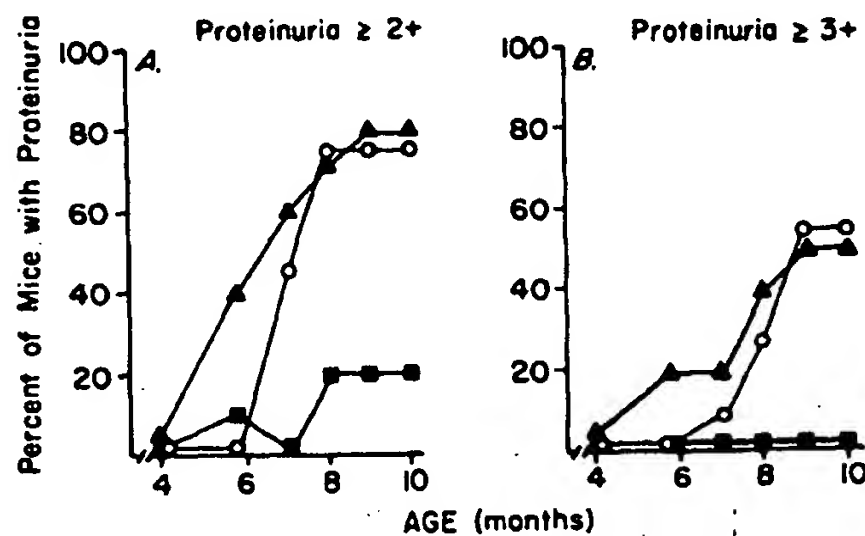


FIGURE 5. Frequency of significant proteinuria in B/W mice treated with saline (O), nonimmune rat IgG (\blacktriangle), or anti-L3T4 mAb (\blacksquare). (A) Percent of mice with proteinuria $\geq 2+$ (100 mg/dl). (B) Percent of mice with proteinuria $\geq 3+$ (300 mg/dl). To reflect accurately the development of renal disease in all mice, each point reflects the current level of proteinuria in surviving mice, as well as the last measurement of proteinuria in deceased mice.

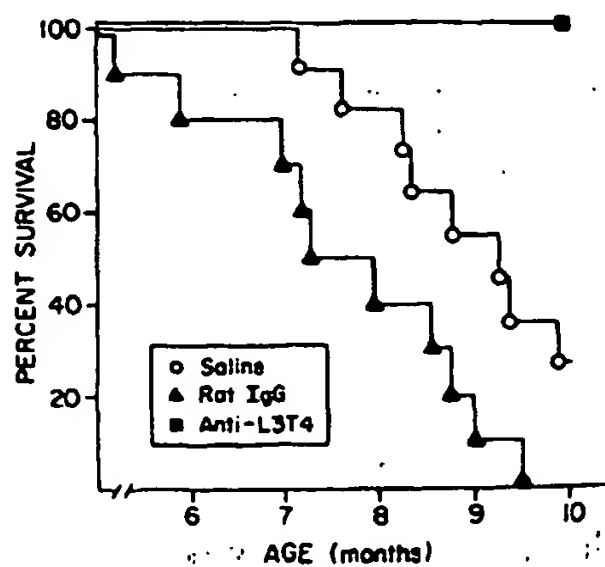


FIGURE 6. Survival in B/W mice treated with saline (O), nonimmune rat IgG (\blacktriangle), or anti-L3T4 mAb (\blacksquare).

mice treated with anti-L3T4 and control mice is strongly significant ($P < 0.001$ compared with the saline control group; $P < 0.00001$ compared with the rat IgG control group; chi-square analysis). The difference in mean survival between the two control groups is also statistically significant ($P < 0.05$; t test), i.e., treatment with nonimmune rat Ig accelerated mortality.

Discussion

To clarify the role of helper T cells in the pathogenesis of autoimmune disease in the B/W model for SLE, we treated female B/W mice with repeated injections of a rat mAb directed against the mouse helper T cell antigen, L3T4. Treatment depleted circulating target cells, reduced autoantibody production, retarded renal disease, and prolonged life. Moreover, treated mice produced little or no host antibodies to the administered rat mAb. These findings provide the first clear demonstration that autoimmune disease in B/W mice is T cell dependent. They further suggest that mAb directed against specific helper T cell antigens may be effective in the treatment of certain autoimmune diseases, and that the therapeutic benefit may be achieved without provoking an undesirable, and potentially hazardous, immune response to the mAb.

For two decades, the B/W strain has been studied (32, 33) as a model for SLE in humans. Early studies (34–37) focused attention on T cell abnormalities in B/W mice, emphasizing defects in suppressor T cell number and function. Recent studies, however, have cast doubt on the significance of these T cell abnormalities. For example, neonatal thymectomy has little effect on the course of autoimmunity in B/W mice (38), but elimination of a specific subset of B cells prevents autoimmune disease (39). Other studies, demonstrating B cell abnormalities, and indicating that B cell abnormalities may precede demonstrable T cell abnormalities in B/W mice, have suggested that autoimmunity in these mice may reflect a primary B cell defect, and that T cell defects may be secondary phenomena (40–43). Our results demonstrate conclusively that T cells, specifically helper T cells, play an important role in the pathogenesis of autoimmune disease in B/W mice. If primary defects occur in other cell types, particularly B lymphocytes, these cells nonetheless require helper T cells for the expression of disease.

It has been postulated that autoimmunity in B/W mice might reflect selective loss of suppressor T cells (36, 37), perhaps as a consequence of the action of natural thymocytotoxic antibodies (44, 45). Our findings in untreated B/W mice show that, although progressive T lymphocytopenia occurs, there is no selective effect on suppressor (Lyt-2⁺) T cells. Rather, both major T cell subsets are comparably reduced, and the L3T4/Lyt-2 ratio is unchanged. There is, thus, no numerical imbalance between helper and suppressor/cytotoxic T cells, although functional differences cannot be excluded.

The L3T4 molecule is expressed on a distinct subpopulation of T cells previously referred to descriptively as helper T cells. More precisely, L3T4 identifies T cells that respond to MHA II on antigen-presenting cells (24–27). The functional importance of the L3T4 molecule is demonstrated by the observation that mAb against L3T4 blocks in vitro T cell responses to MHA II (25–27). The beneficial effects of anti-L3T4 in B/W mice may, therefore, be due not only to target cell depletion, but also to direct inhibition of T cell responses that

are dependent on the recognition of MHA II. The latter mechanism would imply that autoimmunity, like immunity to foreign antigens, requires a T cell response to MHA II. Our results are compatible with this possibility, but they do not prove it. We previously (22) treated B/W mice with mAb against Thy-1.2, an antigen expressed on all T cells. Although treatment reduced circulating L3T4⁺ T cells by 75%, there was a comparable reduction in Lyt-2⁺ T cells, and no improvement in autoimmunity. The failure of anti-Thy-1.2 to improve autoimmunity despite depletion of L3T4⁺ cells suggests either that: (a) regulation of autoimmunity in B/W mice involves a balance between L3T4⁺ cells and Lyt-2⁺ cells that is not disturbed by treatment with anti-Thy-1.2; or (b) successful treatment with anti-L3T4 requires functional impairment of the L3T4 molecule.

An important observation in our present studies was the relative lack of host immunity to the anti-L3T4 antibody. Previous attempts to use mAb as therapeutic agents in humans and in mice have been complicated by the development of a host immune response to the mAb (4, 9, 16, 17, 22). In B/W mice treated with rat anti-Thy-1.2 mAb (subclass IgG2b), the development of antibody to rat Ig was associated with accelerated mortality, even though autoimmunity was not affected (22). In autoimmune BXSB mice, treatment with anti-Thy-1.2 caused fatal anaphylaxis (22). The current study reemphasizes the potential hazards of treatment with foreign Ig, because administration of purified nonimmune rat IgG significantly reduced survival. In this context, the weak host immune response to rat anti-L3T4 mAb (subclass IgG2b) takes on added significance. The ability to administer anti-L3T4 without generating high titers of host antibody to rat Ig may not only maximize the therapeutic effect of anti-L3T4, it may also minimize the risks associated with the host immune response to foreign protein.

The lack of host immunity to repeated injections of anti-L3T4 is the first demonstration that anti-L3T4 can interrupt the immune response *in vivo*. It remains to be determined if anti-L3T4 will block the *in vivo* response to foreign antigens other than itself, although it has been demonstrated (25) that anti-L3T4 can block *in vitro* responses to foreign antigens that are presented in association with MHA II. Immune recognition of MHA II can also be blocked both *in vitro* and *in vivo* by mAb against MHA II (46–49). One group of researchers (50–52) have treated murine models for several autoimmune diseases with mAb against MHA II. This treatment was effective in experimental autoimmune encephalitis (50) and experimentally induced myasthenia gravis (51). It also increased survival in B/W mice, even though it did not reduce autoantibody production (52). In mice, MHA II are expressed on macrophages, B cells, and activated T cells (53), but it is not known whether successful treatment with mAb against MHA II is associated with depletion of these cell types. In humans, MHA II are expressed not only on certain lymphoid cells, but also on blood vessel endothelial cells (54). This may account for the severe toxicity of mAb against MHA II in monkeys (55), which may complicate the use of anti-MHA II in humans. Unlike MHA II, L3T4 appears to be restricted in its expression to a distinct subpopulation of T lymphocytes and T lymphocyte precursors (24). Therefore, anti-L3T4 mAb may provide an alternative to the use of anti-MHA II mAb that would be more selective in its effect on the immune system. The human homologue for the

L3T4 antigen has been identified (23), and mAb against this antigen have already been used to prolong renal allograft survival in nonhuman primates (19).

Treatment of B/W mice with anti-L3T4 had profound effects on the immune system that raise questions about the immune competence of treated mice. The reduction in L3T4⁺ T cells creates an imbalance between T cell subpopulations that resembles the distribution of T cell subsets in acquired immunodeficiency syndrome (AIDS) in people (56). The beneficial reduction in antibodies to double-stranded DNA and antibodies to rat Ig may reflect a more generalized impairment of humoral immunity. Our studies do not establish the significance of these alterations in cellular and humoral immunity, but they do provide some preliminary insight. Despite a 10-fold reduction in autoantibody concentration, treatment with anti-L3T4 did not significantly depress Ig levels. Moreover, treated mice did not develop infectious complications, even though they were housed without special precautions in our main animal colony. Studies are currently in progress in our laboratory to determine the precise effects of treatment with anti-L3T4 on immune competence in normal and autoimmune mice.

The immunologic abnormalities in B/W mice closely parallel the immunologic abnormalities underlying SLE in humans. T lymphocytopenia, impaired suppressor T cell function, reduced lymphokine production, and B cell hyperactivity all occur in humans with SLE as well as in B/W mice, but the significance of these abnormalities remains controversial (32, 57-60). There is no fixed abnormality in the relative proportion of circulating T cell subsets in either B/W mice or humans with SLE (61), but our results make it clear that this does not exclude the possibility that T cells regulate autoimmunity in SLE. The similarities between SLE in humans and SLE in B/W mice suggest that they may share common pathogenetic mechanisms. Our findings in B/W mice therefore support the hypothesis that autoimmunity in people with SLE is regulated by T cells. This hypothesis is consistent with recent studies indicating that total lymphoid irradiation (TLI) improves lupus nephritis in humans (62-64). Although the effect of TLI on lymphocyte subsets in SLE has not yet been determined, TLI causes prolonged depletion and impaired function of Leu-3/T4⁺ cells in people with rheumatoid arthritis (65-67). These observations add weight to the possibility that treatment with mAb against Leu-3/T4 would favorably influence the course of autoimmune disease in people with SLE. However, we believe that such treatment would be premature until the effects of anti-L3T4 on normal immune function have been thoroughly investigated.

Summary

Autoimmune NZB/NZW mice were treated with weekly injections of monoclonal antibody (mAb) to L3T4, an antigen expressed on a distinct subpopulation of T cells that respond to class II major histocompatibility antigens. Treatment with anti-L3T4 depleted circulating target cells, reduced autoantibody production, retarded renal disease, and prolonged life relative to control mice treated either with saline or with purified nonimmune rat IgG. These findings establish that autoimmune disease in NZB/NZW mice is regulated by T cells. In contrast to mice treated with nonimmune rat IgG, mice treated with rat anti-L3T4 mAb

developed little or no antibody to rat Ig. Thus, the benefits of treatment with anti-L3T4 were achieved while minimizing the risks associated with a host immune response to therapy. This study raises the possibility that treatment with mAb against Leu-3/T4, the human homologue for L3T4, might be effective in the treatment of certain autoimmune diseases in people.

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SUCCESSFUL TREATMENT OF AUTOIMMUNITY IN NZB/NZW F₁ MICE WITH MONOCLONAL ANTIBODY TO L3T4

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The advent of hybridoma technology has rekindled hopes of using antibodies as specific therapeutic agents (1-4). Several reports have focused attention on the potential use of monoclonal antibodies (mAb)¹ either as antitumor agents (4-15) or as immunosuppressive agents designed to facilitate organ transplantation (16-21). Early therapeutic trials in humans have demonstrated that certain mAb can be used to retard the progression of lymphoid malignancy (10, 11) or to reverse renal allograft rejection (17, 18). However, in most cases, the beneficial effects of treatment have been transient (4, 12-17) due in part to the development of host immunity to the administered mAb (4, 9, 16, 17).

Recently, (22) we treated three murine models for systemic lupus erythematosus (SLE) with biweekly injections of rat anti-T cell (anti-Thy-1.2) mAb in order to examine the role of T cells in the pathogenesis of murine lupus and to explore the possibility of using antilymphocyte mAb to treat autoimmunity. Treatment with anti-Thy-1.2 substantially reduced circulating T cells in all three strains (MRL/lpr, NZB/NZW F₁, BXSB), despite development of antibodies to rat Ig. Therapy was beneficial in MRL/lpr mice. It reduced autoantibody production, retarded renal disease, and markedly prolonged life. In contrast, treatment did not reduce autoimmunity in NZB/NZW F₁ (B/W) mice, and it caused fatal anaphylaxis in BXSB mice. These findings demonstrate that antilymphocyte mAb can serve as specific probes to examine the cells that contribute to autoimmunity. The results in MRL/lpr mice illustrate the potential therapeutic value of antilymphocyte mAb when a pathogenic cell subset can be identified. However, the results in BXSB mice emphasize equally the potential hazards of the host immune response to treatment with mAb.

The development of a new rat mAb (GK1.5) reactive with mouse helper T cells provides an opportunity for a more selective approach to the study of T cell regulation of autoimmunity in murine lupus. This antibody recognizes a glycoprotein antigen, designated L3T4, that is the mouse homologue for the human T cell antigen termed Leu-3 or T4 (23). Like Leu-3/T4 in humans, L3T4

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¹ Abbreviations used in this paper: BCG, bovine gamma globulin; BSA, bovine serum albumin; BUN, blood urea nitrogen; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; MHA II, class II major histocompatibility antigens; SLE, systemic lupus erythematosus; TLI, total lymphoid irradiation.

participates in the T cell response to class II major histocompatibility antigens (MHA II) on antigen-presenting cells (24–28). Antibody to L3T4 inhibits most MHA II-dependent T cell responses in vitro (25–27). Therefore, administration of mAb against L3T4 might alter immune responses in vivo and thereby influence the course of autoimmune disease. To test this hypothesis, we treated B/W female mice with weekly injections of GK1.5 (anti-L3T4) beginning at age 4 mo. Treatment produced a sustained reduction in circulating T cells expressing L3T4. It also dramatically reduced autoantibody concentrations, retarded renal disease, and prolonged life. Mice treated with rat anti-L3T4 mAb developed little or no antibody to the administered rat Ig. In contrast, control mice treated with purified nonimmune rat IgG developed high titers of antibody to rat Ig. These findings establish that autoimmune disease in B/W mice is T cell-dependent. They further demonstrate that: (a) mAb directed against helper T cells can significantly improve autoimmune disease in B/W mice; and (b) the beneficial effects of treatment with anti-L3T4 may not be complicated by the development of a host immune response to therapy.

Materials and Methods

Mice. B/W mice were bred in our colony at the San Francisco Veterans Administration Medical Center from NZB females, purchased from The Jackson Laboratory, Bar Harbor, ME, and NZW males purchased from Simonsen Laboratory, Gilroy, CA.

Treatment Regimen. 10 mice were treated with a rat IgG2b mAb against L3T4, designated GK1.5 (28). The hybridoma used to produce GK1.5 was generously provided by Dr. Frank W. Fitch, University of Chicago. Antibody to L3T4 was harvested as ascites from sublethally irradiated BALB/c mice, partially purified by ammonium sulfate precipitation, dialyzed against phosphate-buffered saline, and quantified by protein electrophoresis and measurement of optical density. Treatment consisted of an initial intravenous injection of 2 mg of anti-L3T4, followed by weekly intraperitoneal injections (2 mg per mouse). In preliminary studies, we determined that an intravenous injection of 2 mg of anti-L3T4 produces saturation binding of circulating and splenic target cells, and substantial, though incomplete, binding of L3T4⁺ lymph node cells and thymocytes (unpublished data). Two control groups were also studied: one group (10 mice) received weekly 2-mg injections of chromatographically purified rat IgG (Cappel Laboratories, Cochranville, PA); the other group (11 mice) received saline.

Fluorescence Analysis of Lymphocyte Subpopulations. Peripheral blood was obtained from individual mice by retroorbital bleeding into heparinized pipettes. Mononuclear cells were separated by centrifugation over Lympholyte-M (Cedarlane Laboratories, Ontario, Canada), and quantified using a Coulter ZBI cell counter (Coulter Electronics, Inc., Hialeah, FL). Mononuclear cell subsets were identified by staining with fluorescein-conjugated mAb, as described previously (29). The antibodies used for analysis included: anti-Thy-1.2 (30-H12) to identify all T cells, anti-L3T4 (GK1.5) and anti-Lyt-2 (53-6) to identify the major T cell subsets, and anti-ThB (53-9) to identify B cells. Fluorescein-conjugated mouse anti-rat kappa chain mAb (MAR 18.5; B-D FACS Systems, Sunnyvale, CA) was used to detect anti-L3T4 on target cell surfaces. Except as noted, fluorescence analysis was always performed immediately before treatment, i.e., 1 wk after the previous injection.

Assessment of Renal Disease. Blood urea nitrogen (BUN) was determined on samples from individual mice using a CentrifChem System 400 (Union Carbide, Danbury, CT). Proteinuria was measured colorimetrically by the use of Albustix (Miles Laboratories Inc., Elkhart, IN). This produces an approximation of proteinuria as follows: trace, 10 mg/dl; 1+, 30 mg/dl; 2+, 100 mg/dl; 3+, 300 mg/dl; 4+, 1,000 mg/dl. All measurements of proteinuria were performed by an observer who had no knowledge of the given treatment.

Measurement of Serum Antibodies to Double-stranded DNA. Anti-DNA antibody concentrations were assessed by a solid phase enzyme-linked immunosorbent assay (ELISA)

derived from the method of Fish and Ziff (30). Immulon II polyvinyl microtitration trays were coated sequentially with a 50 $\mu\text{g/ml}$ solution of poly-L-lysine (Sigma Chemical Co., St. Louis, MO) in 0.1 M Tris-HCl buffer, pH 7.3 (75 $\mu\text{g/well}$), a 20 $\mu\text{g/ml}$ solution of poly(dA-dT) (Sigma Chemical Co.) in Tris buffer (75 $\mu\text{l/well}$), and a 2% solution of bovine serum albumin (BSA) (Sigma Chemical Co.) in Tris buffer (250 $\mu\text{l/well}$). Serum samples that had been serially diluted in Tris buffer containing 1% BSA and 2% bovine gamma globulin (BGG) (Sigma Chemical Co.) were then incubated at 75 $\mu\text{l/well}$ for 1.5 h at room temperature. The wells were then washed five times and incubated with peroxidase-conjugated goat anti-mouse Ig that had been extensively absorbed with rat Ig (0.3 $\mu\text{g/well}$; Cappel Laboratories). After incubation at room temperature for 1 h, the wells were washed five times and treated in the dark for 10 min with o-phenylenediamine (100 $\mu\text{l/well}$). The enzymatic reaction was stopped with 2 N H₂SO₄ (200 $\mu\text{l/well}$), and absorbance was measured at 490 nm. Serum from adult MRL/lpr and C57BL/6 mice served as positive and negative controls, respectively. The specificity of the assay was demonstrated using wells that had not been coated with poly(dA-dT).

Measurement of Serum Antibodies to Rat Ig. Antibodies to rat Ig were measured by an ELISA technique similar to the one described above. Briefly, Immulon II polyvinyl microtitration trays were coated with rat Ig mAb (0.5 μg 30-H12/well) followed by 2% BSA in 0.1 M Tris-HCl buffer. Serum samples were serially diluted in Tris buffer containing 1% BSA and 2% BGG, then incubated at 75 $\mu\text{l/well}$ for 1.5 h at room temperature. The wells were then washed, incubated with peroxidase-conjugated goat anti-mouse Ig that had been absorbed with rat Ig, washed again, and then treated in the dark with o-phenylenediamine exactly as described above. Mouse anti-rat kappa chain mAb (MAR 18.5) and normal mouse serum served as positive and negative controls, respectively.

Measurement of Serum IgM and IgG Levels. Serum concentrations of mouse IgM and IgG were measured by ELISA. Immulon II polyvinyl microtitration trays were coated with a 10 $\mu\text{g/ml}$ solution of either goat anti-mouse IgG or goat anti-mouse IgM (both, Cappel Laboratories), followed by 2% BSA in 0.1 M Tris-HCl buffer. Serum samples were serially diluted in Tris buffer containing 1% BSA and 2% BGG, then incubated at 75 $\mu\text{l/well}$ for 1.5 h at room temperature. The wells were then washed, incubated with peroxidase-conjugated goat anti-mouse Ig that had been absorbed with rat Ig, washed again, and then treated in the dark with o-phenylenediamine, as described above. Purified mouse IgM and IgG (Miles Laboratories) were used to generate standard curves and document the specificity of the assay.

Results

Effect of Age on Circulating Lymphocyte Subpopulations in Untreated B/W Mice. In control B/W mice treated with saline, there was a spontaneous age-dependent reduction in circulating T lymphocytes (Fig. 1, A and B). The number of circulating B lymphocytes remained relatively constant (Fig. 1C). Although T cell counts fell by ~50% between 4 and 8 mo of age, L3T4⁺ cells and Lyt-2⁺ cells were comparably affected and, therefore, the L3T4/Lyt-2 (helper/suppressor) ratio remained constant (Fig. 1D). Treatment with weekly injections of nonimmune rat IgG did not affect the spontaneous progressive changes in circulating lymphocyte subpopulations in B/W mice.

Effect of Treatment With Anti-L3T4 mAb on Circulating Lymphocyte Subpopulations. Treatment of B/W mice with weekly injections of anti-L3T4 mAb produced a dramatic and sustained reduction in circulating L3T4⁺ lymphocytes (Fig. 1A). Circulating L3T4⁺ cells were reduced by 30% 24 h after the first injection, by 90% 1 wk later, and by 95% thereafter. Fluorescence analysis of circulating lymphocytes using mouse anti-rat kappa chain mAb demonstrated persistence of administered antibody on the few remaining target cells 7 d after

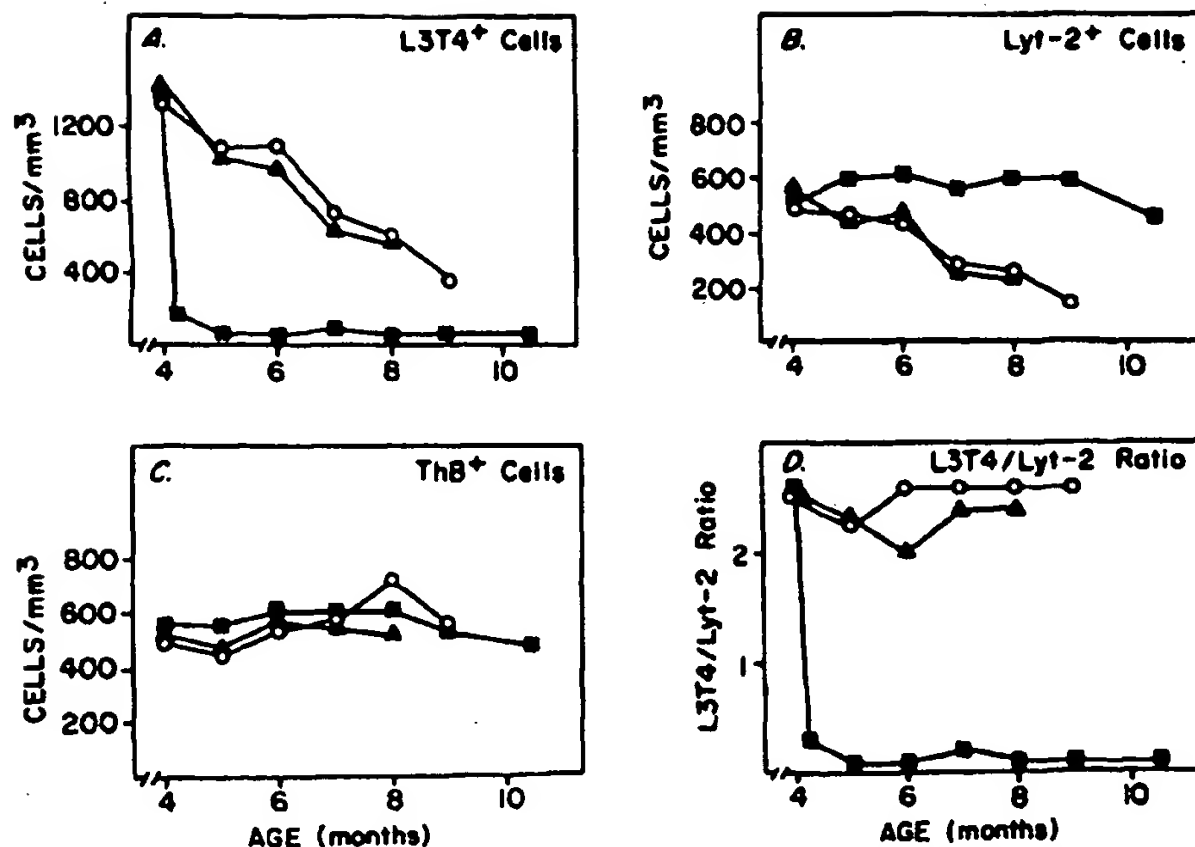


FIGURE 1. Circulating lymphocyte subpopulations in B/W mice treated with weekly injections of saline (O), nonimmune rat IgG (Δ), or anti-L3T4 mAb (■) beginning at age 4 mo. Helper T cells, suppressor/cytotoxic T cells, and B cells were identified using fluorescein-conjugated anti-L3T4 mAb (A), anti-Lyt-2 (B), and anti-Thy B (C), respectively. The relative proportion of L3T4⁺ and Lyt-2⁺ T cells is shown in D.

injection. Staining with fluorescein-conjugated anti-Thy-1.2 mAb and anti-Lyt-2 established that, after 1 wk of therapy, >90% of circulating T cells in treated mice expressed Lyt-2, indicating that the reduction in L3T4⁺ T cells reflected target cell depletion rather than antigen modulation. The effect of treatment with anti-L3T4 was specific. The number of circulating B cells and Lyt-2⁺ T cells remained stable in treated mice (Fig. 1, B and C). The mean L3T4/Lyt-2 ratio in treated mice was 0.1:1, compared with 2.6:1, in untreated mice.

Host Immune Response to Treatment. Control mice treated with nonimmune rat IgG all developed high titers of antibody to rat Ig (Fig. 2). Host antibodies were detectable 7 d after the first injection of rat IgG and rapidly rose to a peak mean titer of 1:4,500. In contrast, only 3 of 10 mice treated with rat anti-L3T4 mAb developed antibodies to rat Ig. In these mice, antibodies to rat Ig first appeared after 2–3 mo of therapy and were present only in relatively low titer (<1:400). 7 of the 10 treated mice never developed detectable antibody to rat Ig. This is in striking contrast to our previous observation that B/W mice treated with rat anti-Thy-1.2 mAb of the same isotype as anti-L3T4 all develop high titers of anti-rat Ig (22).

Anti-L3T4 Reduces Autoimmunity. Treatment of B/W mice with anti-L3T4 mAb prevented development of high titers of antibody to double-stranded DNA (Fig. 3). Although small amounts of anti-DNA antibody appeared within the first

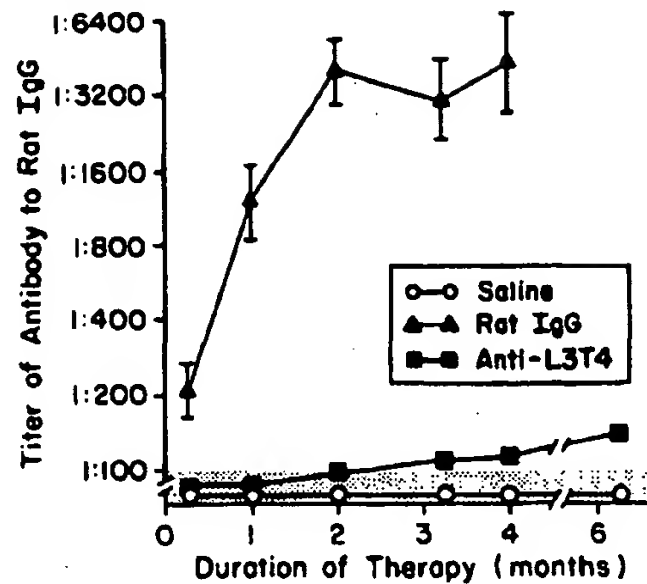


FIGURE 2. Geometric mean titer (\pm SEM) of antibodies to rat Ig in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■). The shaded area indicates assay results using normal mouse sera as negative controls.

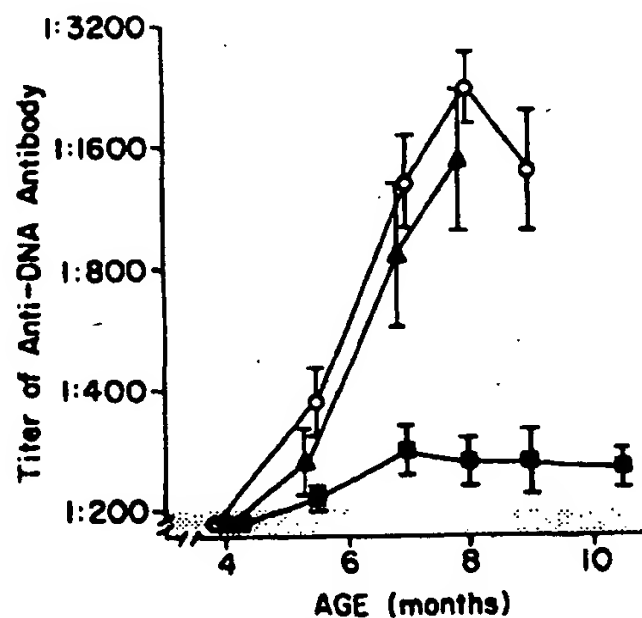


FIGURE 3. Geometric mean titer (\pm SEM) of antibody to double-stranded DNA in B/W mice treated with saline (○), nonimmune rat IgG (▲), or anti-L3T4 mAb (■). The shaded area indicates the titer of anti-DNA antibody (<1:200) in normal (C57BL/6) mice.

2 mo of therapy with anti-L3T4, the titers of anti-DNA antibody were markedly lower in treated mice than in control mice, and the titers remained low throughout the duration of therapy. At age 8 mo, the geometric mean titer of anti-DNA antibody was $1:2,200 \pm 1.2$ in mice treated with saline, $1:1,500 \pm 1.5$ in mice treated with nonimmune rat Ig, and $1:270 \pm 1.1$ in mice treated with anti-L3T4 ($P < 0.0005$ compared with either control group; Student's *t* test). The reduction in anti-DNA antibody did not reflect a generalized reduction in total Ig levels in treated mice (Table I). After 4 mo of treatment, there was a slight reduction in the mean IgG concentration in treated mice that was not statistically significant.

TABLE I
Effect of Treatment on Ig Concentration

Treatment	IgM*		IgG*	
	4-mo-old	8-mo-old	4-mo-old	8-mo-old
Saline	9.5 ± 0.2	9.2 ± 0.2	15.5 ± 0.2	15.7 ± 0.2
Rat IgG	9.6 ± 0.2	9.6 ± 0.2	15.7 ± 0.3	15.5 ± 0.6
Anti-L3T4	9.6 ± 0.2	10.9 ± 0.2 [‡]	15.5 ± 0.3	15.2 ± 0.3

* Log₂ titer ($\bar{x} \pm$ SEM) as measured by ELISA.

[‡] $P < 0.05$ compared to pretreatment IgM levels and to IgM levels in both control groups.

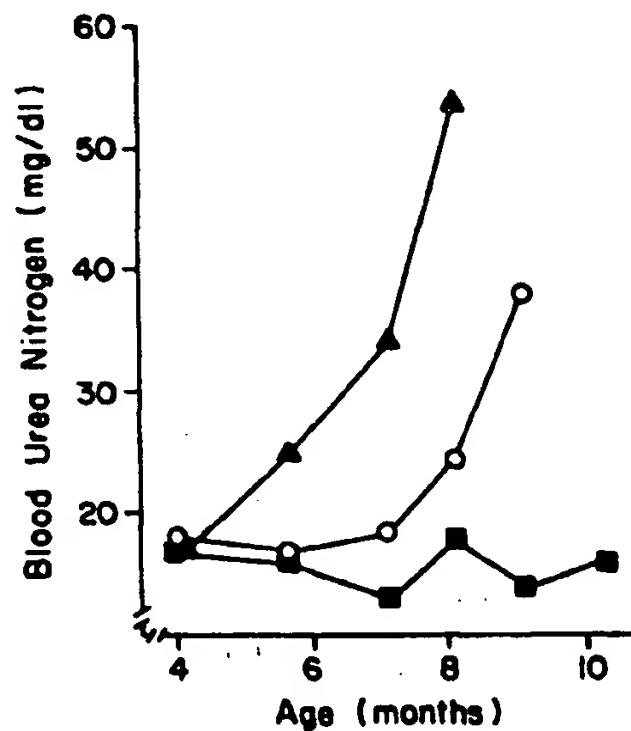


FIGURE 4. Mean BUN in B/W mice treated with saline (O), nonimmune rat IgG (Δ) or anti-L3T4 mAb (■).

However, there was a significant increase in the mean IgM concentration in treated mice compared with pretreatment levels, and with IgM levels in control mice. Increased plasma IgM has been reported previously (31) in association with successful treatment of B/W mice.

Anti-L3T4 Reduces Renal Disease. The reduction in anti-DNA antibody concentration in mice treated with anti-L3T4 was associated with a significant reduction in renal disease. Renal function, as assessed by BUN, deteriorated steadily in both control groups, but was preserved in mice treated with anti-L3T4 (Fig. 4). Renal insufficiency occurred earliest in mice treated with non-immune rat IgG, perhaps as a consequence of the host immune response to rat Ig. At age 7 mo, the mean BUN in mice treated with rat IgG was 34.2 ± 12.1 mg/dl, compared to 18.5 ± 2.7 mg/dl in mice treated with saline ($P < 0.05$; Mann-Whitney U test) and 13.1 ± 0.8 mg/dl in mice treated with anti-L3T4 ($P < 0.01$ compared with rat IgG treatment; $P < 0.05$ compared with saline treatment). By 9 mo, the mean BUN in mice treated with saline rose to 38.0 ± 11.6 mg/dl, compared with 14.3 ± 0.9 mg/dl in mice treated with anti-L3T4 (P

< 0.01). Severe proteinuria ($\geq 3+$) developed in 50–60% of mice in each control group, but in none of the mice treated with anti-L3T4 ($P < 0.05$; chi-square analysis) (Fig. 5). Moderate proteinuria ($2+$) developed in 16 of 21 control mice but in only 2 of 10 treated mice ($P < 0.05$). These two mice were among the three treated mice that developed low titers of antibody to rat Ig, suggesting that the immune response to rat Ig may have contributed to the development of proteinuria.

Anti-L3T4 Prolongs Survival. Treatment with anti-L3T4 mAb dramatically prolonged life (Fig. 6). Median survival was 9.3 mo in mice treated with saline and 7.6 mo in mice treated with rat IgG. In contrast, all of the mice treated with anti-L3T4 are still alive at 10 mo. The difference in 10-mo survival between

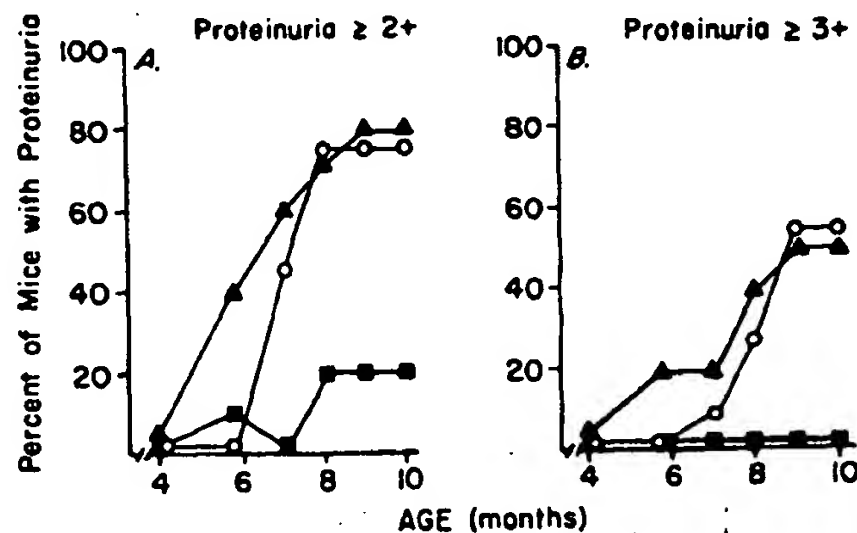


FIGURE 5. Frequency of significant proteinuria in B/W mice treated with saline (O), nonimmune rat IgG (\blacktriangle), or anti-L3T4 mAb (\blacksquare). (A) Percent of mice with proteinuria $\geq 2+$ (100 mg/dl). (B) Percent of mice with proteinuria $\geq 3+$ (300 mg/dl). To reflect accurately the development of renal disease in all mice, each point reflects the current level of proteinuria in surviving mice, as well as the last measurement of proteinuria in deceased mice.

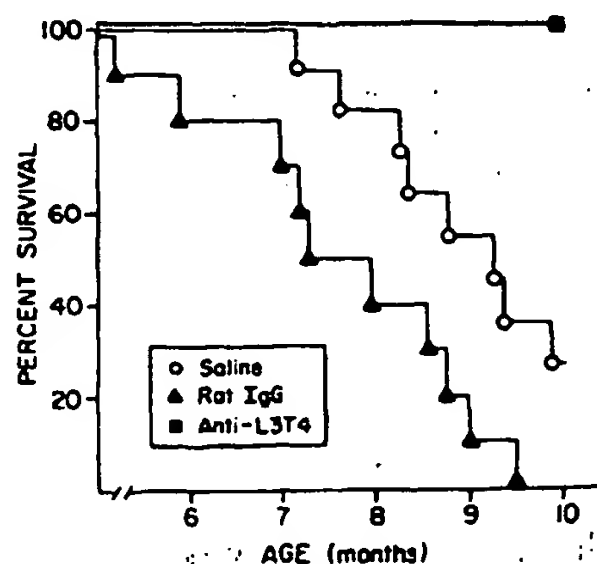


FIGURE 6. Survival in B/W mice treated with saline (O), nonimmune rat IgG (\blacktriangle), or anti-L3T4 mAb (\blacksquare).

mice treated with anti-L3T4 and control mice is strongly significant ($P < 0.001$ compared with the saline control group; $P < 0.00001$ compared with the rat IgG control group; chi-square analysis). The difference in mean survival between the two control groups is also statistically significant ($P < 0.05$; t test), i.e., treatment with nonimmune rat Ig accelerated mortality.

Discussion

To clarify the role of helper T cells in the pathogenesis of autoimmune disease in the B/W model for SLE, we treated female B/W mice with repeated injections of a rat mAb directed against the mouse helper T cell antigen, L3T4. Treatment depleted circulating target cells, reduced autoantibody production, retarded renal disease, and prolonged life. Moreover, treated mice produced little or no host antibodies to the administered rat mAb. These findings provide the first clear demonstration that autoimmune disease in B/W mice is T cell dependent. They further suggest that mAb directed against specific helper T cell antigens may be effective in the treatment of certain autoimmune diseases, and that the therapeutic benefit may be achieved without provoking an undesirable, and potentially hazardous, immune response to the mAb.

For two decades, the B/W strain has been studied (32, 33) as a model for SLE in humans. Early studies (34–37) focused attention on T cell abnormalities in B/W mice, emphasizing defects in suppressor T cell number and function. Recent studies, however, have cast doubt on the significance of these T cell abnormalities. For example, neonatal thymectomy has little effect on the course of autoimmunity in B/W mice (38), but elimination of a specific subset of B cells prevents autoimmune disease (39). Other studies, demonstrating B cell abnormalities, and indicating that B cell abnormalities may precede demonstrable T cell abnormalities in B/W mice, have suggested that autoimmunity in these mice may reflect a primary B cell defect, and that T cell defects may be secondary phenomena (40–43). Our results demonstrate conclusively that T cells, specifically helper T cells, play an important role in the pathogenesis of autoimmune disease in B/W mice. If primary defects occur in other cell types, particularly B lymphocytes, these cells nonetheless require helper T cells for the expression of disease.

It has been postulated that autoimmunity in B/W mice might reflect selective loss of suppressor T cells (36, 37), perhaps as a consequence of the action of natural thymocytotoxic antibodies (44, 45). Our findings in untreated B/W mice show that, although progressive T lymphocytopenia occurs, there is no selective effect on suppressor (Lyt-2⁺) T cells. Rather, both major T cell subsets are comparably reduced, and the L3T4/Lyt-2 ratio is unchanged. There is, thus, no numerical imbalance between helper and suppressor/cytotoxic T cells, although functional differences cannot be excluded.

The L3T4 molecule is expressed on a distinct subpopulation of T cells previously referred to descriptively as helper T cells. More precisely, L3T4 identifies T cells that respond to MHA II on antigen-presenting cells (24–27). The functional importance of the L3T4 molecule is demonstrated by the observation that mAb against L3T4 blocks in vitro T cell responses to MHA II (25–27). The beneficial effects of anti-L3T4 in B/W mice may, therefore, be due not only to target cell depletion, but also to direct inhibition of T cell responses that

are dependent on the recognition of MHA II. The latter mechanism would imply that autoimmunity, like immunity to foreign antigens, requires a T cell response to MHA II. Our results are compatible with this possibility, but they do not prove it. We previously (22) treated B/W mice with mAb against Thy-1.2, an antigen expressed on all T cells. Although treatment reduced circulating L3T4⁺ T cells by 75%, there was a comparable reduction in Lyt-2⁺ T cells, and no improvement in autoimmunity. The failure of anti-Thy-1.2 to improve autoimmunity despite depletion of L3T4⁺ cells suggests either that: (a) regulation of autoimmunity in B/W mice involves a balance between L3T4⁺ cells and Lyt-2⁺ cells that is not disturbed by treatment with anti-Thy-1.2; or (b) successful treatment with anti-L3T4 requires functional impairment of the L3T4 molecule.

An important observation in our present studies was the relative lack of host immunity to the anti-L3T4 antibody. Previous attempts to use mAb as therapeutic agents in humans and in mice have been complicated by the development of a host immune response to the mAb (4, 9, 16, 17, 22). In B/W mice treated with rat anti-Thy-1.2 mAb (subclass IgG2b), the development of antibody to rat Ig was associated with accelerated mortality, even though autoimmunity was not affected (22). In autoimmune BXSB mice, treatment with anti-Thy-1.2 caused fatal anaphylaxis (22). The current study reemphasizes the potential hazards of treatment with foreign Ig, because administration of purified nonimmune rat IgG significantly reduced survival. In this context, the weak host immune response to rat anti-L3T4 mAb (subclass IgG2b) takes on added significance. The ability to administer anti-L3T4 without generating high titers of host antibody to rat Ig may not only maximize the therapeutic effect of anti-L3T4, it may also minimize the risks associated with the host immune response to foreign protein.

The lack of host immunity to repeated injections of anti-L3T4 is the first demonstration that anti-L3T4 can interrupt the immune response *in vivo*. It remains to be determined if anti-L3T4 will block the *in vivo* response to foreign antigens other than itself, although it has been demonstrated (25) that anti-L3T4 can block *in vitro* responses to foreign antigens that are presented in association with MHA II. Immune recognition of MHA II can also be blocked both *in vitro* and *in vivo* by mAb against MHA II (46–49). One group of researchers (50–52) have treated murine models for several autoimmune diseases with mAb against MHA II. This treatment was effective in experimental autoimmune encephalitis (50) and experimentally induced myasthenia gravis (51). It also increased survival in B/W mice, even though it did not reduce autoantibody production (52). In mice, MHA II are expressed on macrophages, B cells, and activated T cells (53), but it is not known whether successful treatment with mAb against MHA II is associated with depletion of these cell types. In humans, MHA II are expressed not only on certain lymphoid cells, but also on blood vessel endothelial cells (54). This may account for the severe toxicity of mAb against MHA II in monkeys (55), which may complicate the use of anti-MHA II in humans. Unlike MHA II, L3T4 appears to be restricted in its expression to a distinct subpopulation of T lymphocytes and T lymphocyte precursors (24). Therefore, anti-L3T4 mAb may provide an alternative to the use of anti-MHA II mAb that would be more selective in its effect on the immune system. The human homologue for the

L3T4 antigen has been identified (23), and mAb against this antigen have already been used to prolong renal allograft survival in nonhuman primates (19).

Treatment of B/W mice with anti-L3T4 had profound effects on the immune system that raise questions about the immune competence of treated mice. The reduction in L3T4⁺ T cells creates an imbalance between T cell subpopulations that resembles the distribution of T cell subsets in acquired immunodeficiency syndrome (AIDS) in people (56). The beneficial reduction in antibodies to double-stranded DNA and antibodies to rat Ig may reflect a more generalized impairment of humoral immunity. Our studies do not establish the significance of these alterations in cellular and humoral immunity, but they do provide some preliminary insight. Despite a 10-fold reduction in autoantibody concentration, treatment with anti-L3T4 did not significantly depress Ig levels. Moreover, treated mice did not develop infectious complications, even though they were housed without special precautions in our main animal colony. Studies are currently in progress in our laboratory to determine the precise effects of treatment with anti-L3T4 on immune competence in normal and autoimmune mice.

The immunologic abnormalities in B/W mice closely parallel the immunologic abnormalities underlying SLE in humans. T lymphocytopenia, impaired suppressor T cell function, reduced lymphokine production, and B cell hyperactivity all occur in humans with SLE as well as in B/W mice, but the significance of these abnormalities remains controversial (32, 57-60). There is no fixed abnormality in the relative proportion of circulating T cell subsets in either B/W mice or humans with SLE (61), but our results make it clear that this does not exclude the possibility that T cells regulate autoimmunity in SLE. The similarities between SLE in humans and SLE in B/W mice suggest that they may share common pathogenetic mechanisms. Our findings in B/W mice therefore support the hypothesis that autoimmunity in people with SLE is regulated by T cells. This hypothesis is consistent with recent studies indicating that total lymphoid irradiation (TLI) improves lupus nephritis in humans (62-64). Although the effect of TLI on lymphocyte subsets in SLE has not yet been determined, TLI causes prolonged depletion and impaired function of Leu-3/T4⁺ cells in people with rheumatoid arthritis (65-67). These observations add weight to the possibility that treatment with mAb against Leu-3/T4 would favorably influence the course of autoimmune disease in people with SLE. However, we believe that such treatment would be premature until the effects of anti-L3T4 on normal immune function have been thoroughly investigated.

Summary

Autoimmune NZB/NZW mice were treated with weekly injections of monoclonal antibody (mAb) to L3T4, an antigen expressed on a distinct subpopulation of T cells that respond to class II major histocompatibility antigens. Treatment with anti-L3T4 depleted circulating target cells, reduced autoantibody production, retarded renal disease, and prolonged life relative to control mice treated either with saline or with purified nonimmune rat IgG. These findings establish that autoimmune disease in NZB/NZW mice is regulated by T cells. In contrast to mice treated with nonimmune rat IgG, mice treated with rat anti-L3T4 mAb

developed little or no antibody to rat Ig. Thus, the benefits of treatment with anti-L3T4 were achieved while minimizing the risks associated with a host immune response to therapy. This study raises the possibility that treatment with mAb against Leu-3/T4, the human homologue for L3T4, might be effective in the treatment of certain autoimmune diseases in people.

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T Cell Subsets and the Recognition of MHC Class

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INTRODUCTION

It is the purpose of this review to summarize our recent observations and those of other researchers on several aspects of T cell subsets as they relate to recognition of major histocompatibility complex (MHC) antigens. These observations have led us to conclude that there are two subsets of T cells which recognize respectively the two classes of MHC antigen (Class 1 and Class 2) and which can be identified by their Lyt (murine) or Leu (human) phenotype. Several features of the T cell recognition of MHC have led us to propose that there are two separate components of this recognition. In one component, T cell molecules (probably related to or identical to the Lyt or Leu molecules) interact with an invariant part(s) of either the Class 1 or Class 2 molecule which is monomorphic for that class. In the second component, the "T cell receptor" (which could either be independent or linked to the class-interaction molecule) recognizes the polymorphic determinants of the same MHC molecule. This recognition would be of the MHC alone in the dual recognition model or of MHC plus non-MHC antigen in the interaction antigen or altered self-model. The three sets of experimental observations, which will be presented below, led to the following conclusions:

- 1) The Lyt or Leu phenotype and susceptibility to blocking with appropriate antibodies of the vast majority of murine and human T cells correlates with the Class of MHC molecule recognized by those T cells.
- 2) Xenogeneic T cells respond only to allele-specific MHC determinants and are not directed to species-specific non-MHC or monomorphic MHC determinants.
- 3) T cells responding across species barriers make the same distinction of MHC Class as do T cells participating in allogeneic or syngeneic interactions.

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EXPERIMENTAL OBSERVATIONS

1. *Lyt and Leu phenotypes and susceptibility to MoAb blocking are associated with recognition of MHC class not with T cell function*

The experiments of Cantor and Boyse (1976) in the mouse had established that in most cases helper T cells express Lyt1 but not Lyt2 molecules while cytotoxic and some suppressor cells express Lyt2 but not Lyt1 molecules. It had also been demonstrated that helper cells recognize allogeneic or are restricted to self Class 2 MHC molecules while cytotoxic T cells recognize allogeneic or are restricted to self Class 1 molecules (for early reviews see Bach et al. 1976, Sprent 1978, Doherty et al. 1976). Thus, it was established that there is a triple correlation in which helper T cells are Lyt1⁺2⁻ and recognize Class 2 MHC antigens while cytotoxic T cells are Lyt1⁻2⁺ and recognize Class 1 MHC antigens.

We showed in our earlier experiments that there can be exceptions to the correlation between T cell function and MHC class in the response to foreign MHC alleles (discussed in Dutton et al. 1978). In an analysis of these exceptions, we were able to show that the cell surface phenotype of the T cell correlates more faithfully with the class of MHC molecule recognized than with the T cell function. In bulk cultures of normal spleen cells, helper T cells that recognized Class 1 K- and D-encoded antigens were Lyt2⁺ while cytotoxic T cells, both in bulk populations and in one cloned line that recognized IA, were either Lyt2-negative (cloned line) or expressed much lower amounts of Lyt2 than CTL directed against K or D allo-antigens (Swain & Panfili 1979, Swain et al. 1979, Swain et al. 1981). We also showed that in the case where there was an unusual correlation between class recognition and function there was no restriction to the usual class of MHC (Swain 1981a).

TABLE I

Lyt 2 Expression and Susceptibility to Anti Lyt 2 Blocking is Associated with Class 1 MHC Recognition by T Cells of Several Functions

MHC Subregion Recognized	Function	Lyt 2 Expression	Anti-Lyt 2 Blocking
<i>Class 1</i>			
	Help (1°)	+++	+++
	IL2 production (2°)	+++	+++
	Cytotoxicity (1° and 2°)	+++	++
<i>Class 2</i>			
	Help (1°)	-	-
	IL2 production (2°)	-	-
	Cytotoxicity (1°)	+/-	+/-
	Cytotoxicity (2°)	+/-	+/-

Based on data from Swain and Panfili (1979), Swain (1981) and Swain, (unpublished) on the allogeneic T cell response.

Class 1 Specif
Expt. 1
K^b→K^{bml}

Expt. 2
K^b→K^{bml}

Expt. 3
D^a→D^d

Class 2 Specif
Expt. 1
I^a→I^k

Expt. 2
I^a→I^k

Expt. 3
H-2^b→H-2^{bxd}

Mls-Specific
Expt. 1
Mls^b→Mls^a

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TABLE II
Reciprocal Blocking of Allo-help by MoAbs to Lyt2 and L3T4

	T Cells	B Cells	MoAb Specificity	PFC/culture
<i>Class 1 Specific</i>				
<i>Expt. 1</i>	—	B6.bml	—	2 (1- 2)
$K^b \rightarrow K^{bml}$	B6	+	—	203 (181-227)
	+	+	L3T4	271 (228-323)
	+	+	Lyt2	24 (20- 28)
	+	+	LFA-1	<2
<i>Expt. 2</i>	—	B6.bml	—	≤2
$K^b \rightarrow K^{bml}$	B6	+	—	452 (381-536)
	+	+	L3T4	351 (308-399)
	+	+	Lyt2	12 (5- 28)
<i>Expt. 3</i>	—	B10.T (6R)	—	<
$D^a \rightarrow D^d$	B10.G	+	—	83 (70- 99)
	+	+	L3T4	60 (56- 64)
	+	+	Lyt2	9 (4- 19)
<i>Class 2 Specific/or Whole Haplotype</i>				
<i>Expt. 1</i>	—	B10.AQR	—	2 (1- 3)
$I^a \rightarrow I^k$	B10.T (6R)	+	—	435 (408-465)
	+	+	L3T4	21 (9- 52)
	+	+	Lyt2	502 (476-531)
<i>Expt. 2</i>	—	B10.AQR	—	<2
$I^a \rightarrow I^k$	B10.T (6R)	+	—	36 (26- 48)
	+	+	L3T4	6 (3- 9)
	+	+	Lyt2	30 (25- 36)
<i>Expt. 3</i>	—	BDF ₁	—	4 (2- 11)
$H-2^b \rightarrow H-2^{bx+d}$	B6	+	—	1,056 (1,024-1,087)
	+	+	L3T4	98 (91-106)
	+	+	Lyt2	626 (556-704)
	+	+	LFA1	3 (2- 5)
<i>Mls-Specific</i>				
<i>Expt. 1</i>	—	D1.LP	—	149 (120-184)
$Mls^b \rightarrow Mls^a$	B6	+	—	614 (442-853)
	+	+	L3T4	45 (35- 57)
	+	+	Lyt2	791 (711-880)
	+	+	LFA-1	11 (7- 17)

In these experiments T-depleted B cells developed PFC in response to SRBC and were helped by allogeneic T cells that differed at either Class 1 or Class 2 loci or at Mls. The monoclonal antibodies were present throughout the 4-day culture period.

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The same correlations could be shown in experiments where Lyt2 monoclonal antibodies were used to block activity. The helper function or IL2 production of cells responding to K or D allo-antigens could be blocked by monoclonal anti-Lyt2, while cytotoxic function specific for IA or whole I region could not be blocked by the same reagent (Swain 1981b). These findings are summarized in Table I. It recently became apparent that Lyt1 is not expressed reciprocally to Lyt2 (Swain & Dutton 1981, Ledbetter et al. 1981) and does not block Class 2 specific function. Thus, at the time these initial experiments were done it was not possible to examine the blocking of T cell responses to Class 2 antigens because of the lack of an appropriate reagent to define a true reciprocal of Lyt2 (but see below).

More recently it has been shown that the same pattern of observations can be documented in humans, mainly in experiments utilizing cloned lines (Engelman et al. 1981, Krensky et al. 1982, Reinherz et al. 1981, Biddison et al. 1982, Meuer et al. 1982). In humans, the marker, Leu 1, would appear to be the equivalent of the mouse Lyt1 that it is expressed to varying degrees on all T cells. Antisera to the Lyt1 and/or Leu 1 do not generally block responses. Leu 2 appears to correspond to Lyt2 (Ledbetter et al. 1981). Leu 3, however, has a reciprocal distribution to Leu 2 and appears to define the T cell subset that recognizes or is restricted to Class 2 MHC antigen. Antibody to Leu 3 has been shown to block the response to Class 2 antigen.

An equivalent marker, L3T4, has now been found in the mouse by Dialynas

TABLE III
Reciprocal Blocking of IL2 Production by MoAbs to Lyt2 and L3T4

Primed Responders	Stimulator B10.T (6R)	Specificity of MoAb	CPM/Culture with 10% Supernatant
None	—	None	68 (60– 77)
<i>Class 1 Reactive</i>			
1. B10.G	+	None	461 (434– 489)
2. B10.G	+	Lyt2	112 (111– 113)
3. B10.G	+	L3T4	560 (546– 574)
4. B10.G	+ Con A	None	971 (802–1,176)
<i>Class 2 Reactive</i>			
5. B10.AQR	+	None	830 (656–1,046)
6. B10.AQR	+	Lyt2	457 (356– 586)
7. B10.AQR	+	L3T4	81 (77– 86)
8. B10.AQR	+ Con A	None	956 (825–1,101)

Primed T cell populations were prepared in response to stimulators allogeneic at Class 1 or Class 2 MHC. The primed T cells were re-stimulated with the appropriate stimulators in the presence or absence of monoclonal antibodies to Lyt2 or L3T4 and the 24-h IL2 production assayed on an IL2 dependent cell line as previously described (Swain et al. 1983).

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and his colleagues, and is defined by the monoclonal antibody GK1.5 (Dialynas et al. 1983). We were then able to complete our analysis in the mouse and report here that GK1.5 (anti-L3T4) can block allogeneic help and lymphokine production elicited by Class 2 but not Class 1 MHC. The results, using bulk cultures, are illustrated in Tables II and III. It can be seen that antibody to L3T4 was able to inhibit both allo-help (Table II) and the production of IL2 (Table III) to I region antigens and MIs, but not to those encoded by K and D subregions. It is of interest that monoclonal antibody to LFA 1 (an unrelated cell surface antigen) which had been previously reported to block killing to K and D (Davignon et al. 1981) showed no class recognition selectivity and blocked helper responses to both I and K/D. The impressive correlation of Lyt phenotype, blocking with Lyt antibodies and recognition of MHC subregion in the mouse is summarized in Table IV.

2. Xenogeneic T cell responses recognize only allelic specificities

Lindahl and Bach (1975, 1976) showed that the response of human T cells to mouse lymphocytes was allele-specific and that there was no response to the non-polymorphic 'mouse' MHC determinants.

We have confirmed and extended these observations in our own study of human anti-mouse mixed lymphocyte responses (Swain et al. 1983). In these studies we showed that T cells stimulated with mouse lymphocytes in a primary *in vitro* response could only be re-stimulated by MHC compatible mouse lymphocytes in a secondary response. Responses re-stimulated with whole H-2 compatible or Class 1-compatible stimulators resulted in the development of

TABLE IV
Reciprocal Blocking of Allogeneic T Cell Reactivity to Class 1 and Class 2 MHC Subregion Antigens

MHC Subregion Recognized	Function	Anti-Lyt2 Blocking	Anti-L3T4 Blocking	Anti-LFA-1 Blocking
<i>Class 1</i>				
	Help (1°)	+++	-	++++
	IL2 production (2°)	+++	-	++++
	Cytotoxicity	+++	-	++++*
<i>Class 2</i>				
	Help (1°)	-	+++	++++
	IL2 production (2°)	-	+++	++++
	Cytotoxicity (1° and 2°)	+/-	++	++++

* Data from Davignon et al. 1981. Based on data in this review and in Swain, Dialynas, Fitch and English, manuscript in preparation.

TABLE V
Specificity of Human T Cells Responsive to Mouse Stimulators

Restimulator or target genes shared with primary stimulator	Response measured (Degree)	
	IL2 production	Target lysis
Whole MHC \pm Background	+++	+++
Background	-	-
Class 1 \pm Background (K or D)	-	+++
Class 2 \pm Background	++++	-

This data is a summary of our experience with human anti-mouse responses. Details and experimental methods can be found in Swain et al. (1983). Primary cultures of 4×10^7 human PBLs and 1×10^7 mouse splenic T-depleted, mitogentreated stimulators were cultured for 7-10 days in 20 ml in flasks. Cells were recultured with mouse 2° stimulator cells from mice sharing MHC and/or background genes with those used in the primary culture. IL2 production was determined by assaying the 24-h culture supernatants of 2° cultures. Cytotoxicity was determined on Con A plus LPS blasted mouse spleen cells.

CTL activity specific for the relevant Class 1 allele, while responses restimulated with Class 2-compatible (to responder) splenic non-T cells generated IL2 production. These results are summarized in Table V.

Two features of these responses can be emphasized.

- 1) The response was all directed to allele-specific (polymorphic) determinants, i.e., there was no measurable response to any shared mouse MHC (monomorphic) determinants or to other non-MHC (background) determinants.
- 2) The human T cells were able to make the same functional distinction between murine Class 1 and Class 2 MHC as in the allogeneic and syngeneic response in that the appropriate T cell function was correlated with the MHC class recognized.

3. T cells which distinguish MHC class across species barriers are appropriately blocked by MoAbs to Leu antigens

As already indicated in the previous section, the response of human T cells to mouse Class 2 antigens resulted in IL2 production while CTL were generated in response to Class 1 antigens. The fact that human T cells distinguished mouse Class 1 and Class 2 structures in analogous manner to that in responses within the species was further illustrated by blocking experiments with anti-Leu 2 and anti-Leu 3 reagents. The cytotoxic response to K and D was blocked by anti-Leu 2 while the IL2 production elicited by restimulation with Class 1 was blocked by anti-Leu 3. These findings (Swain et al. 1983) are illustrated in Table VI.

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TABLE VI
Susceptibility to Anti-Leu 2a and 3a Blocking of 2° Human and Mouse Responses

Specificity of Anti-Leu Antibody	IL2 Production	% specific Cytotoxicity
	ΔCPM	
None	768	22
Leu 2a	804	8
Leu 3a	-8	26

Primary cultures of human PBLs were set up against B10.A (3R) mouse, T-depleted splenic stimulators. After 10 days cultures were re-stimulated with B10.A (3R) T-depleted spleen cells. Supernatants were harvested after 18 h to determine IL2 production and specific cytotoxicity was determined after 4 days on LPS plus Con A B10.A (3R) blasts. Further details in Swain et al. 1983.

DISCUSSION

1. Implications of experimental observations

Before going on to a broader discussion of T cell recognition, the significance of the apparent involvement of Lyt and Leu antigens in T cell recognition of MHC class will be analyzed.

The correlation of the expression of the Lyt2 antigen with recognition of Class 1 MHC is very impressive when bulk populations of normal or short term *in vitro* primed T cells from mouse spleen are used in the functional assays used in our experiments. These include unprimed and *in vitro* primed allo-help and IL2 production (Swain 1981b) and *in vivo* primed cytotoxic responses (Swain 1981b). In contrast, published results in unprimed cytotoxic responses in the mouse are somewhat less consistent, for instance: while Vidovic et al. (1981) found most Class 2-specific killers came from Lyt2⁺ precursors, Miller & Stutman (1982) found anti-Lyt2 blocking of both Class 1- and Class 2-directed cytotoxic T cells. In our experiments, Class 2-specific precursors of cytotoxic effectors expressed less Lyt2 than those directed to Class 1, but a certain proportion were, nonetheless, removed by anti-Lyt2 and C treatment. Furthermore, among hybridomas and cytotoxic clones are found examples of Class 1-specific cytotoxic effectors which do not appear to express Lyt2 (MacDonald et al. 1982) and which are not always blocked by anti-Lyt2 reagents (MacDonald et al. 1982). There are several possible explanations for these deviations from the Lyt-MHC subregion rule.

The first explanation, only applicable to the bulk CTL responses, is that some T cells in putative I region only different cultures, may really be specific for non-K or -D Class 2 antigens including L, R, Qa and possibly others. This is not unlikely in bulk cultures and indeed among *cloned* I-specific (mouse) and DR-

specific (human) lines or clones where the specificity has been determined, a very good correlation has been found, i.e., an absence of Lyt2 and Leu 2 markers and presence of L3T4 and Leu 3 (Swain et al. 1981, Dialynas et al. 1983, Krensky et al. 1982, Meuer et al. 1982).

However, this explanation does not deal with T cell clones which can be shown to be specific for Class 1 and yet lack and/or are not blocked by, anti Lyt2. The exceptions in these cases put important constraints on the possible roles for Lyt2. Long term lines and clones, however, may not always accurately reflect normal phenotypes. Nonetheless, it is clear that Lyt2 molecules are *not always* involved in T cell recognition of Class 1 MHC.

In order to interpret these results, several more facets of blocking with monoclonal and polyclonal antibodies are relevant.

(1) Very few reagents specific for T cell surface markers have been found which are able to block T cell function despite extensive attempts to find such reagents (reviewed in Immunol. Rev. 68). The first of these, and one of the two most commonly found, are reagents to Lyt2 which were first found to block cytotoxic function by Shinohara and Sachs (1979) and Nakayama et al. (1979). Since those initial reports, a wide variety of studies have confirmed and extended these observations (reviewed in Immunol. Rev. 68). The only other commonly found antibodies which block function are those against the high molecular weight antigens termed LFA-1 (Davignon et al. 1981, Pierres et al. 1982) which are large proteins (94-108K) expressed on all subsets of T cells (as well as B cells). Antibody to LFA-1 blocks multiple T cell functions (all tested) regardless of specificity (Davignon et al. 1981, Pierres et al. 1982, and results in this report).

(2) Anti-Lyt2 reagents appear to block cytotoxicity (both induction and effector function) at the very early phase of precursor/effector interaction with the target (Fan et al. 1980, MacDonald et al. 1982, Goldstein et al. 1982, Hollander 1982, Springer et al. 1982). Furthermore, lectins such as Con A overcome both the target specificity of killing and blocking with anti Lyt2. Allo-help and IL2 production are also blocked at the induction stage (Swain 1981b) and Con A also overcomes both the specificity and blocking (Swain 1981b). In all cases the T cell is clearly the target. Both of these facets suggest anti-Lyt2 blocks recognition.

(3) Finally, molecules of similar structure are found in many species (mouse, human, rat) and in all cases antisera and monoclonal antibodies to these structures have been found which block function.

Taken together, the above three sets of observations make a persuasive, though somewhat circumstantial case that the molecules in question (Lyt2 and L3T4; Leu 2, Leu 3) play an important role in T cell response to MHC antigen. The most likely role from these and our own observations would be in recognition of MHC class.

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obligatory. One way to reconcile these two phenomena is to suggest that the Ly- and Leu-encoded molecules interact with monomorphic class-specific determinants. We suggest that this interaction contributes to or facilitates the interaction of the clonally distributed antigen-specific part of the receptor(s) which participate(s) in recognition of polymorphic determinants on MHC and of nominal antigen. It is thus possible that high affinity specific receptors or lectin-facilitated binding would not require the facilitatory class-specific interaction. In fact, data from MacDonald et al. (1982) includes one instance in which anti-Lyt2 failed to block the original antigen-induced cytotoxic activity but did block a cross-reaction of that same clone consistent with a requirement for Lyt2 involvement only when a, presumably, lower affinity interaction occurs. Also the same group previously demonstrated that primed cytotoxic effectors derived from peritoneal exudate were less susceptible to anti-Lyt2 blocking than those derived from spleen (MacDonald et al. 1981).

2. Speculations on T cell recognition

The experimental observations that we would like to consider in further discussion of how T cells recognize MHC include not only those summarized above but a number of earlier basic features of the T cell interaction with MHC. They are listed and discussed in sequence.

(1) Approximately 1% of the T cell population is activated in response to a foreign MHC allele. The exact figure is not important, but it has been clear from the earliest experiments (Dutton 1965, Nisbet et al. 1969) that perhaps the majority of all T cells must be reactive to one or another foreign allele. This feature was neither explained or rendered less remarkable when it was revealed that all T cell responses to non-MHC antigens are restricted to self-MHC (Katz et al. 1973, Zinkernagel & Doherty 1975). There would appear to be only two explanations: either that the recognition structures consisted of a limited set of specificities restricted to the recognition of allogeneic MHC or that the repertoire was "open" (and large) but there is a "bias" towards MHC that allows a single MHC allele antigen to capture a large portion of the T cell repertoire.

(2) The T cell recognition of MHC is limited to the polymorphic parts of the molecule. This is seen both in the self-MHC restricted response to non-MHC antigen (Zinkernagel & Doherty 1979) and in the response to allogeneic MHC. It might be argued that the lack of a response to the invariant part of the molecule was due to the elimination of such reactivity by a mechanism of tolerance-induction to self-components. The finding of a similar preoccupation with polymorphic determinants in xenogeneic responses discussed above and seen in several other xenogeneic situations, however, would seem to rule out an explanation based on self-tolerance, unless the non-polymorphic regions were extremely conserved between species, which, from the data available, does not

seem to be the case. Again there would seem to be a constraint either on the repertoire of receptor structures itself and/or upon the way it functions; e.g., some sort of obligatory interaction with MHC polymorphic regions.

(3) T cell clones specific for non-MHC antigens restricted to self-MHC (self+X) often cross-react with a foreign MHC allele (originally described by Sredni & Schwartz 1981). The frequency of such reactivity in some cases is so high as to suggest that perhaps all T cells react with one or another of the species' polymorphic structures of MHC. Further interpretation of this is heavily weighted by whether one considers a two-receptor or one-receptor model for T cell recognition. Several pieces of information presented in this review seem relevant here.

(4) T cells that recognize Class 1 MHC antigens carry different cell-surface molecules from those that recognize Class 2 MHC antigens. (5) This recognition operates across species barriers and (6) the consequences of recognition can be blocked by the presence of antibody to the appropriate cell-surface marker. We consider that the most likely conclusions to be drawn from this are: first, that the recognition of MHC class is carried out independently from the recognition of MHC polymorphic determinants (and the non-MHC antigen); and second, that the relevant cell surface structures are in some way associated with this recognition as outlined earlier.

3. A model for the T cell receptor

We have, therefore, proposed a model (Swain et al. 1983) in which the recognition of MHC class is separated from the recognition of MHC poly-

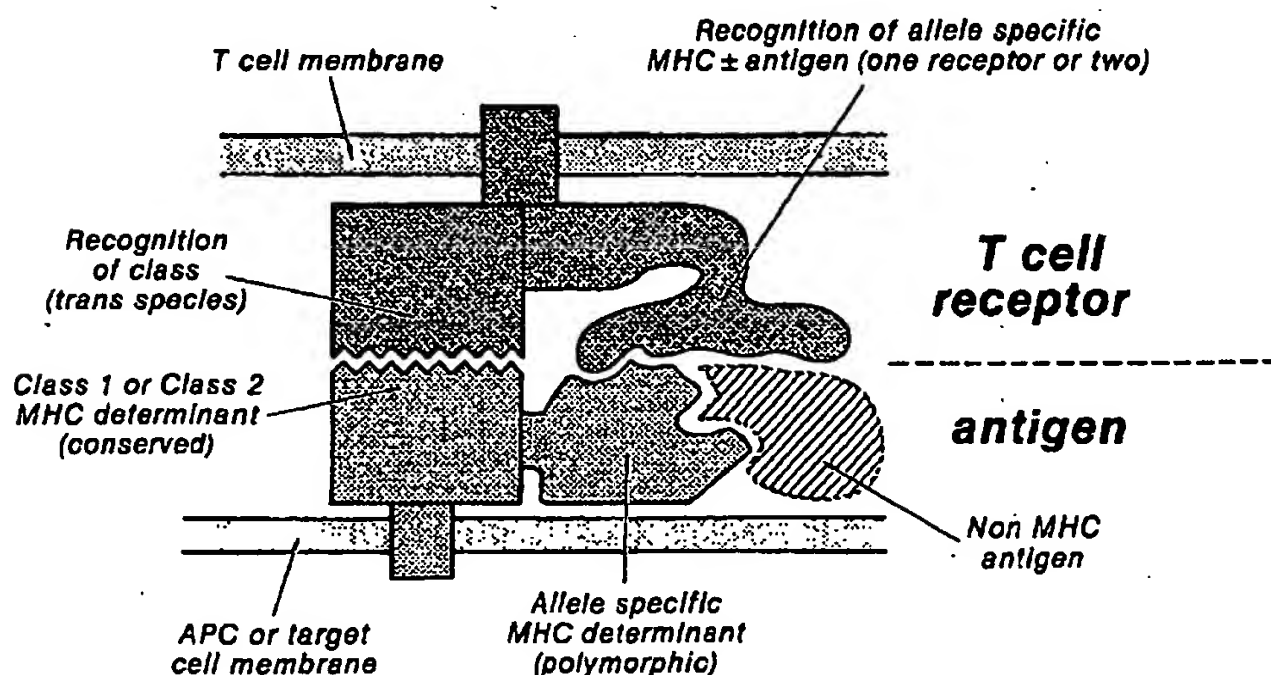


Figure 1. Hypothesis for recognition of MHC class and polymorphic determinants by T cells. This model was previously published in *J. Exp. Med.* 157, 720.

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morphic determinants and non-MHC antigen (see Figure 1). We further suggest that the recognition of the class-specific part of the MHC molecule 'positions' the receptor so that it can only interact with area where the polymorphic variations occur. The concept conveyed by the model can be drawn in many ways and the version presented here is merely one presentation of the principles involved. We have drawn the separate components of the model joined together with a single attachment to the membrane. The two components could be independently attached and need not be associated with one another before the interaction occurs or even then. In its essence, this model is not affected by whether MHC and non-MHC are recognized by one or two recognition events.

SUMMARY

We have presented and/or briefly reviewed data which indicates that there are two T cell subsets which interact respectively with the two Classes (1 and 2) of MHC antigen and which can be identified by the Ly (mouse) or Leu (human) molecules that they express. This correlation, and the large body of (largely) circumstantial but still quite convincing data, suggests that these Ly and Leu molecules play a very important role in T cell responses by actually interacting with monomorphic MHC class specific determinants. We suggest that this interaction facilitates and possibly helps direct the binding of the T cell receptor to polymorphic MHC determinants and antigen. In this model T cell "recognition" of MHC and antigen consists of several independent but connected interactions of T cell surface structure with MHC molecules and antigen on antigen-presenting cells or targets.

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Immunological Reviews

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Correlation of Human Surface Parameters

L. L. LANIER
N. L. WARNE

Recognition of antigens through the dendritic lymphoid cell surface is a complex process. These antigen receptors, with the associated sorting, have been divided into subsets. Of particular interest is the surface antigen of the cell. All antigens are as diverse as the negatively selected biochemical pathways. It is possible to understand the function by a

Several methods using monoclonal selection, can identify subsets of cells

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T Cell Subsets and the Recognition of MHC Class

SUSAN L. SWAIN

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INTRODUCTION

It is the purpose of this review to summarize our recent observations and those of other researchers on several aspects of T cell subsets as they relate to recognition of major histocompatibility complex (MHC) antigens. These observations have led us to conclude that there are two subsets of T cells which recognize respectively the two classes of MHC antigen (Class 1 and Class 2) and which can be identified by their Lyt (murine) or Leu (human) phenotype. Several features of the T cell recognition of MHC have led us to propose that there are two separate components of this recognition. In one component, T cell molecules (probably related to or identical to the Lyt or Leu molecules) interact with an invariant part(s) of either the Class 1 or Class 2 molecule which is monomorphic for that class. In the second component, the "T cell receptor" (which could either be independent or linked to the class-interaction molecule) recognizes the polymorphic determinants of the same MHC molecule. This recognition would be of the MHC alone in the dual recognition model or of MHC plus non-MHC antigen in the interaction antigen or altered self-model. The three sets of experimental observations, which will be presented below, led to the following conclusions:

- 1) The Lyt or Leu phenotype and susceptibility to blocking with appropriate antibodies of the vast majority of murine and human T cells correlates with the Class of MHC molecule recognized by those T cells.
- 2) Xenogeneic T cells respond only to allele-specific MHC determinants and are not directed to species-specific non-MHC or monomorphic MHC determinants.
- 3) T cells responding across species barriers make the same distinction of MHC Class as do T cells participating in allogeneic or syngeneic interactions.

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EXPERIMENTAL OBSERVATIONS

1. *Lyt and Leu phenotypes and susceptibility to MoAb blocking are associated with recognition of MHC class not with T cell function*

The experiments of Cantor and Boyse (1976) in the mouse had established that in most cases helper T cells express Lyt1 but not Lyt2 molecules while cytotoxic and some suppressor cells express Lyt2 but not Lyt1 molecules. It had also been demonstrated that helper cells recognize allogeneic or are restricted to self Class 2 MHC molecules while cytotoxic T cells recognize allogeneic or are restricted to self Class 1 molecules (for early reviews see Bach et al. 1976, Sprent 1978, Doherty et al. 1976). Thus, it was established that there is a triple correlation in which helper T cells are Lyt1^+2^- and recognize Class 2 MHC antigens while cytotoxic T cells are Lyt1^-2^+ and recognize Class 1 MHC antigens.

We showed in our earlier experiments that there can be exceptions to the correlation between T cell function and MHC class in the response to foreign MHC alleles (discussed in Dutton et al. 1978). In an analysis of these exceptions, we were able to show that the cell surface phenotype of the T cell correlates more faithfully with the class of MHC molecule recognized than with the T cell function. In bulk cultures of normal spleen cells, helper T cells that recognized Class 1 K- and D-encoded antigens were Lyt2^+ while cytotoxic T cells, both in bulk populations and in one cloned line that recognized IA, were either Lyt2 -negative (cloned line) or expressed much lower amounts of Lyt2 than CTL directed against K or D allo-antigens (Swain & Panfili 1979, Swain et al. 1979, Swain et al. 1981). We also showed that in the case where there was an unusual correlation between class recognition and function there was no restriction to the usual class of MHC (Swain 1981a).

TABLE I

Lyt 2 Expression and Susceptibility to Anti Lyt 2 Blocking is Associated with Class 1 MHC Recognition by T Cells of Several Functions

MHC Subregion Recognized	Function	Lyt 2 Expression	Anti-Lyt 2 Blocking
<i>Class 1</i>			
	Help (1°)	+++	+++
	IL2 production (2°)	+++	+++
	Cytotoxicity (1° and 2°)	+++	++
<i>Class 2</i>			
	Help (1°)	—	—
	IL2 production (2°)	—	—
	Cytotoxicity (1°)	+/-	+/-
	Cytotoxicity (2°)	+/-	+/-

Based on data from Swain and Panfili (1979), Swain (1981) and Swain, (unpublished) on the allogeneic T cell response.

Class 1 Specif
Expt. 1
 $\text{K}^b \rightarrow \text{K}^{\text{bm1}}$

Expt. 2
 $\text{K}^b \rightarrow \text{K}^{\text{mb1}}$

Expt. 3
 $\text{D}^a \rightarrow \text{D}^d$

Class 2 Specif.
Expt. 1
 $\text{I}^a \rightarrow \text{I}^k$

Expt. 2
 $\text{I}^a \rightarrow \text{I}^k$

Expt. 3
 $\text{H-2}^b \rightarrow \text{H-2}^{\text{bnd}}$

Mls-Specific
Expt. 1
 $\text{Mls}^b \rightarrow \text{Mls}^a$

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TABLE II
Reciprocal Blocking of Allo-help by MoAbs to Lyt2 and L3T4

	T Cells	B Cells	MoAb Specificity	PFC/culture
<i>Class 1 Specific</i>				
<i>Expt. 1</i>	—	B6.bml	—	2 (1- 2)
$K^b \rightarrow K^{bml}$	B6	+	—	203 (181-227)
	+	+	L3T4	271 (228-323)
	+	+	Lyt2	24 (20- 28)
	+	+	LFA-1	<2
<i>Expt. 2</i>	—	B6.bml	—	≤2
$K^b \rightarrow K^{bml}$	B6	+	—	452 (381-536)
	+	+	L3T4	351 (308-399)
	+	+	Lyt2	12 (5- 28)
<i>Expt. 3</i>	—	B10.T (6R)	—	<
$D^a \rightarrow D^d$	B10.G	+	—	83 (70- 99)
	+	+	L3T4	60 (56- 64)
	+	+	Lyt2	9 (4- 19)
<i>Class 2 Specific/or Whole Haplotype</i>				
<i>Expt. 1</i>	—	B10.AQR	—	2 (1- 3)
$I^a \rightarrow I^k$	B10.T (6R)	+	—	435 (408-465)
	+	+	L3T4	21 (9- 52)
	+	+	Lyt2	502 (476-531)
<i>Expt. 2</i>	—	B10.AQR	—	<2
$I^a \rightarrow I^k$	B10.T (6R)	+	—	36 (26- 48)
	+	+	L3T4	6 (3- 9)
	+	+	Lyt2	30 (25- 36)
<i>Expt. 3</i>	—	BDF ₁	—	4 (2- 11)
$H-2^b \rightarrow H-2^{bx}$	B6	+	—	1,056 (1,024-1,087)
	+	+	L3T4	98 (91-106)
	+	+	Lyt2	626 (556-704)
	+	+	LFA1	3 (2- 5)
<i>Mls-Specific</i>				
<i>Expt. 1</i>	—	D1.LP	—	149 (120-184)
$Mls^b \rightarrow Mls^a$	B6	+	—	614 (442-853)
	+	+	L3T4	45 (35- 57)
	+	+	Lyt2	791 (711-880)
	+	+	LFA-1	11 (7- 17)

In these experiments T-depleted B cells developed PFC in response to SRBC and were helped by allogeneic T cells that differed at either Class 1 or Class 2 loci or at Mls. The monoclonal antibodies were present throughout the 4-day culture period.

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The same correlations could be shown in experiments where Lyt2 monoclonal antibodies were used to block activity. The helper function or IL2 production of cells responding to K or D allo-antigens could be blocked by monoclonal anti-Lyt2, while cytotoxic function specific for IA or whole I region could not be blocked by the same reagent (Swain 1981b). These findings are summarized in Table I. It recently became apparent that Lyt1 is not expressed reciprocally to Lyt2 (Swain & Dutton 1981, Ledbetter et al. 1981) and does not block Class 2 specific function. Thus, at the time these initial experiments were done it was not possible to examine the blocking of T cell responses to Class 2 antigens because of the lack of an appropriate reagent to define a true reciprocal of Lyt2 (but see below).

More recently it has been shown that the same pattern of observations can be documented in humans, mainly in experiments utilizing cloned lines (Engelman et al. 1981, Krensky et al. 1982, Reinherz et al. 1981, Biddison et al. 1982, Meuer et al. 1982). In humans, the marker, Leu 1, would appear to be the equivalent of the mouse Lyt1 that it is expressed to varying degrees on all T cells. Antisera to the Lyt1 and/or Leu 1 do not generally block responses. Leu 2 appears to correspond to Lyt2 (Ledbetter et al. 1981). Leu 3, however, has a reciprocal distribution to Leu 2 and appears to define the T cell subset that recognizes or is restricted to Class 2 MHC antigen. Antibody to Leu 3 has been shown to block the response to Class 2 antigen.

An equivalent marker, L3T4, has now been found in the mouse by Dialynas

TABLE III
Reciprocal Blocking of IL2 Production by MoAbs to Lyt2 and L3T4

Primed Responders	Stimulator B10.T (6R)	Specificity of MoAb	CPM/Culture with 10% Supernatant
None	—	None	68 (60–77)
<i>Class 1 Reactive</i>			
1. B10.G	+	None	461 (434–489)
2. B10.G	+	Lyt2	112 (111–113)
3. B10.G	+	L3T4	560 (546–574)
4. B10.G	+Con A	None	971 (802–1,176)
<i>Class 2 Reactive</i>			
5. B10.AQR	+	None	830 (656–1,046)
6. B10.AQR	+	Lyt2	457 (356–586)
7. B10.AQR	+	L3T4	81 (77–86)
8. B10.AQR	+Con A	None	956 (825–1,101)

Primed T cell populations were prepared in response to stimulators allogeneic at Class 1 or Class 2 MHC. The primed T cells were re-stimulated with the appropriate stimulators in the presence or absence of monoclonal antibodies to Lyt2 or L3T4 and the 24-h IL2 production assayed on an IL2 dependent cell line as previously described (Swain et al. 1983).

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and his colleagues, and is defined by the monoclonal antibody GK1.5 (Dialynas et al. 1983). We were then able to complete our analysis in the mouse and report here that GK1.5 (anti-L3T4) can block allogeneic help and lymphokine production elicited by Class 2 but not Class 1 MHC. The results, using bulk cultures, are illustrated in Tables II and III. It can be seen that antibody to L3T4 was able to inhibit both allo-help (Table II) and the production of IL2 (Table III) to I region antigens and Mls, but not to those encoded by K and D subregions. It is of interest that monoclonal antibody to LFA 1 (an unrelated cell surface antigen) which had been previously reported to block killing to K and D (Davignon et al. 1981) showed no class recognition selectivity and blocked helper responses to both I and K/D. The impressive correlation of Lyt phenotype, blocking with Lyt antibodies and recognition of MHC subregion in the mouse is summarized in Table IV.

2. Xenogeneic T cell responses recognize only allelic specificities

Lindahl and Bach (1975, 1976) showed that the response of human T cells to mouse lymphocytes was allele-specific and that there was no response to the non-polymorphic 'mouse' MHC determinants.

We have confirmed and extended these observations in our own study of human anti-mouse mixed lymphocyte responses (Swain et al. 1983). In these studies we showed that T cells stimulated with mouse lymphocytes in a primary *in vitro* response could only be re-stimulated by MHC compatible mouse lymphocytes in a secondary response. Responses re-stimulated with whole H-2 compatible or Class 1-compatible stimulators resulted in the development of

TABLE IV
Reciprocal Blocking of Allogeneic T Cell Reactivity to Class 1 and Class 2 MHC Subregion Antigens

MHC Subregion Recognized	Function	Anti-Lyt2 Blocking	Anti-L3T4 Blocking	Anti-LFA-1 Blocking
<i>Class 1</i>				
	Help (1°)	+++	-	++++
	IL2 production (2°)	+++	-	++++
	Cytotoxicity	+++	-	++++*
<i>Class 2</i>				
	Help (1°)	-	+++	++++
	IL2 production (2°)	-	+++	++++
	Cytotoxicity (1° and 2°)	+/-	++	++++

* Data from Davignon et al. 1981. Based on data in this review and in Swain, Dialynas, Fitch and English, manuscript in preparation.

TABLE V
Specificity of Human T Cells Responsive to Mouse Stimulators

Restimulator or target genes shared with primary stimulator	Response measured (Degree)	
	IL2 production	Target lysis
Whole MHC \pm Background	+++	+++
Background	—	—
Class 1 \pm Background (K or D)	—	+++
Class 2 \pm Background	++++	—

This data is a summary of our experience with human anti-mouse responses. Details and experimental methods can be found in Swain et al. (1983). Primary cultures of 4×10^7 human PBLs and 1×10^7 mouse splenic T-depleted, mitogentreated stimulators were cultured for 7–10 days in 20 ml in flasks. Cells were recultured with mouse 2° stimulator cells from mice sharing MHC and/or background genes with those used in the primary culture. IL2 production was determined by assaying the 24-h culture supernatants of 2° cultures. Cytotoxicity was determined on Con A plus LPS blasted mouse spleen cells.

CTL activity specific for the relevant Class 1 allele, while responses restimulated with Class 2-compatible (to responder) splenic non-T cells generated IL2 production. These results are summarized in Table V.

Two features of these responses can be emphasized.

- 1) The response was all directed to allele-specific (polymorphic) determinants, i.e., there was no measurable response to any shared mouse MHC (monomorphic) determinants or to other non-MHC (background) determinants.
- 2) The human T cells were able to make the same functional distinction between murine Class 1 and Class 2 MHC as in the allogeneic and syngeneic response in that the appropriate T cell function was correlated with the MHC class recognized.

3. T cells which distinguish MHC class across species barriers are appropriately blocked by MoAbs to Leu antigens

As already indicated in the previous section, the response of human T cells to mouse Class 2 antigens resulted in IL2 production while CTL were generated in response to Class 1 antigens. The fact that human T cells distinguished mouse Class 1 and Class 2 structures in analogous manner to that in responses within the species was further illustrated by blocking experiments with anti-Leu 2 and anti-Leu 3 reagents. The cytotoxic response to K and D was blocked by anti-Leu 2 while the IL2 production elicited by restimulation with Class 1 was blocked by anti-Leu 3. These findings (Swain et al. 1983) are illustrated in Table VI.

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Primary culture stimulators. Supernatants was determined al. 1983.

1. Implications

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TABLE VI
Susceptibility to Anti-Leu 2a and 3a Blocking of 2° Human and Mouse Responses

Specificity of Anti-Leu Antibody	IL2 Production	% specific Cytotoxicity
	ΔCPM	
None	768	22
Leu 2a	804	8
Leu 3a	-8	26

Primary cultures of human PBLs were set up against B10.A (3R) mouse, T-depleted splenic stimulators. After 10 days cultures were re-stimulated with B10.A (3R) T-depleted spleen cells. Supernatants were harvested after 18 h to determine IL2 production and specific cytotoxicity was determined after 4 days on LPS plus Con A B10.A (3R) blasts. Further details in Swain et al. 1983.

DISCUSSION

1. Implications of experimental observations

Before going on to a broader discussion of T cell recognition, the significance of the apparent involvement of Lyt and Leu antigens in T cell recognition of MHC class will be analyzed.

The correlation of the expression of the Lyt2 antigen with recognition of Class 1 MHC is very impressive when bulk populations of normal or short term *in vitro* primed T cells from mouse spleen are used in the functional assays used in our experiments. These include unprimed and *in vitro* primed allo-help and IL2 production (Swain 1981b) and *in vivo* primed cytotoxic responses (Swain 1981b). In contrast, published results in unprimed cytotoxic responses in the mouse are somewhat less consistent, for instance: while Vidovic et al. (1981) found most Class 2-specific killers came from Lyt2⁺ precursors, Miller & Stutman (1982) found anti-Lyt2 blocking of both Class 1- and Class 2-directed cytotoxic T cells. In our experiments, Class 2-specific precursors of cytotoxic effectors expressed less Lyt2 than those directed to Class 1, but a certain proportion were, nonetheless, removed by anti-Lyt2 and C treatment. Furthermore, among hybridomas and cytotoxic clones are found examples of Class 1-specific cytotoxic effectors which do not appear to express Lyt2 (MacDonald et al. 1982) and which are not always blocked by anti-Lyt2 reagents (MacDonald et al. 1982). There are several possible explanations for these deviations from the Lyt-MHC subregion rule.

The first explanation, only applicable to the bulk CTL responses, is that some T cells in putative I region only different cultures, may really be specific for non-K or -D Class 2 antigens including L, R, Qa and possibly others. This is not unlikely in bulk cultures and indeed among *cloned* I-specific (mouse) and DR-

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specific (human) lines or clones where the specificity has been determined, a very good correlation has been found, i.e., an absence of Lyt2 and Leu 2 markers and presence of L3T4 and Leu 3 (Swain et al. 1981, Dialynas et al. 1983, Krensky et al. 1982, Meuer et al. 1982).

However, this explanation does not deal with T cell clones which can be shown to be specific for Class 1 and yet lack and/or are not blocked by, anti Lyt2. The exceptions in these cases put important constraints on the possible roles for Lyt2. Long term lines and clones, however, may not always accurately reflect normal phenotypes. Nonetheless, it is clear that Lyt2 molecules are *not always* involved in T cell recognition of Class 1 MHC.

In order to interpret these results, several more facets of blocking with monoclonal and polyclonal antibodies are relevant.

(1) Very few reagents specific for T cell surface markers have been found which are able to block T cell function despite extensive attempts to find such reagents (reviewed in Immunol. Rev. 68). The first of these, and one of the two most commonly found, are reagents to Lyt2 which were first found to block cytotoxic function by Shinohara and Sachs (1979) and Nakayama et al. (1979). Since those initial reports, a wide variety of studies have confirmed and extended these observations (reviewed in Immunol. Rev. 68). The only other commonly found antibodies which block function are those against the high molecular weight antigens termed LFA-1 (Davignon et al. 1981, Pierres et al. 1982) which are large proteins (94-108K) expressed on all subsets of T cells (as well as B cells). Antibody to LFA-1 blocks multiple T cell functions (all tested) regardless of specificity (Davignon et al. 1981, Pierres et al. 1982, and results in this report).

(2) Anti-Lyt2 reagents appear to block cytotoxicity (both induction and effector function) at the very early phase of precursor/effector interaction with the target (Fan et al. 1980, MacDonald et al. 1982, Goldstein et al. 1982, Hollander 1982, Springer et al. 1982). Furthermore, lectins such as Con A overcome both the target specificity of killing and blocking with anti Lyt2. Allo-help and IL2 production are also blocked at the induction stage (Swain 1981b) and Con A also overcomes both the specificity and blocking (Swain 1981b). In all cases the T cell is clearly the target. Both of these facets suggest anti-Lyt2 blocks recognition.

(3) Finally, molecules of similar structure are found in many species (mouse, human, rat) and in all cases antisera and monoclonal antibodies to these structures have been found which block function.

Taken together, the above three sets of observations make a persuasive, though somewhat circumstantial case that the molecules in question (Lyt2 and L3T4; Leu 2, Leu 3) play an important role in T cell response to MHC antigen. The most likely role from these and our own observations would be in recognition of MHC class.

The negative data suggests that whatever the role of Lyt2, it is not always

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2. Speculation

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obligatory. One way to reconcile these two phenomena is to suggest that the Ly- and Leu-encoded molecules interact with monomorphic class-specific determinants. We suggest that this interaction contributes to or facilitates the interaction of the clonally distributed antigen-specific part of the receptor(s) which participate(s) in recognition of polymorphic determinants on MHC and of nominal antigen. It is thus possible that high affinity specific receptors or lectin-facilitated binding would not require the facilitatory class-specific interaction. In fact, data from MacDonald et al. (1982) includes one instance in which anti-Lyt2 failed to block the original antigen-induced cytotoxic activity but did block a cross-reaction of that same clone consistent with a requirement for Lyt2 involvement only when a, presumably, lower affinity interaction occurs. Also the same group previously demonstrated that primed cytotoxic effectors derived from peritoneal exudate were less susceptible to anti-Lyt2 blocking than those derived from spleen (MacDonald et al. 1981).

2. Speculations on T cell recognition

The experimental observations that we would like to consider in further discussion of how T cells recognize MHC include not only those summarized above but a number of earlier basic features of the T cell interaction with MHC. They are listed and discussed in sequence.

(1) Approximately 1% of the T cell population is activated in response to a foreign MHC allele. The exact figure is not important, but it has been clear from the earliest experiments (Dutton 1965, Nisbet et al. 1969) that perhaps the majority of all T cells must be reactive to one or another foreign allele. This feature was neither explained or rendered less remarkable when it was revealed that all T cell responses to non-MHC antigens are restricted to self-MHC (Katz et al. 1973, Zinkernagel & Doherty 1975). There would appear to be only two explanations: either that the recognition structures consisted of a limited set of specificities restricted to the recognition of allogeneic MHC or that the repertoire was "open" (and large) but there is a "bias" towards MHC that allows a single MHC allele antigen to capture a large portion of the T cell repertoire.

(2) The T cell recognition of MHC is limited to the polymorphic parts of the molecule. This is seen both in the self-MHC restricted response to non-MHC antigen (Zinkernagel & Doherty 1979) and in the response to allogeneic MHC. It might be argued that the lack of a response to the invariant part of the molecule was due to the elimination of such reactivity by a mechanism of tolerance-induction to self-components. The finding of a similar preoccupation with polymorphic determinants in xenogeneic responses discussed above and seen in several other xenogeneic situations, however, would seem to rule out an explanation based on self-tolerance, unless the non-polymorphic regions were extremely conserved between species, which, from the data available, does not

seem to be the case. Again there would seem to be a constraint either on the repertoire of receptor structures itself and/or upon the way it functions; e.g., some sort of obligatory interaction with MHC polymorphic regions.

(3) T cell clones specific for non-MHC antigens restricted to self-MHC (self+X) often cross-react with a foreign MHC allele (originally described by Sredni & Schwartz 1981). The frequency of such reactivity in some cases is so high as to suggest that perhaps all T cells react with one or another of the species' polymorphic structures of MHC. Further interpretation of this is heavily weighted by whether one considers a two-receptor or one-receptor model for T cell recognition. Several pieces of information presented in this review seem relevant here.

(4) T cells that recognize Class 1 MHC antigens carry different cell-surface molecules from those that recognize Class 2 MHC antigens. (5) This recognition operates across species barriers and (6) the consequences of recognition can be blocked by the presence of antibody to the appropriate cell-surface marker. We consider that the most likely conclusions to be drawn from this are: first, that the recognition of MHC class is carried out independently from the recognition of MHC polymorphic determinants (and the non-MHC antigen); and second, that the relevant cell surface structures are in some way associated with this recognition as outlined earlier.

3. A model for the T cell receptor

We have, therefore, proposed a model (Swain et al. 1983) in which the recognition of MHC class is separated from the recognition of MHC poly-

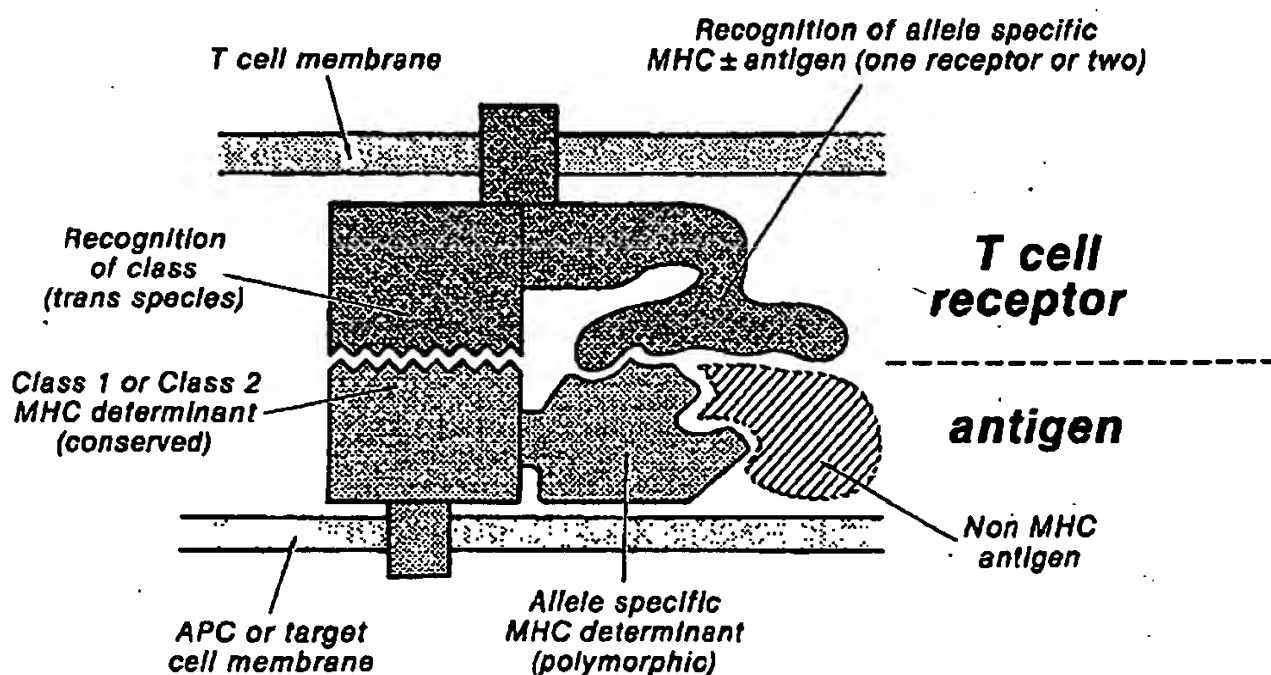


Figure 1. Hypothesis for recognition of MHC class and polymorphic determinants by T cells. This model was previously published in *J. Exp. Med.* 157, 720.

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morphic determinants and non-MHC antigen (see Figure 1). We further suggest that the recognition of the class-specific part of the MHC molecule 'positions' the receptor so that it can only interact with area where the polymorphic variations occur. The concept conveyed by the model can be drawn in many ways and the version presented here is merely one presentation of the principles involved. We have drawn the separate components of the model joined together with a single attachment to the membrane. The two components could be independently attached and need not be associated with one another before the interaction occurs or even then. In its essence, this model is not affected by whether MHC and non-MHC are recognized by one or two recognition events.

SUMMARY

We have presented and/or briefly reviewed data which indicates that there are two T cell subsets which interact respectively with the two Classes (1 and 2) of MHC antigen and which can be identified by the Ly (mouse) or Leu (human) molecules that they express. This correlation, and the large body of (largely) circumstantial but still quite convincing data, suggests that these Ly and Leu molecules play a very important role in T cell responses by actually interacting with monomorphic MHC class specific determinants. We suggest that this interaction facilitates and possibly helps direct the binding of the T cell receptor to polymorphic MHC determinants and antigen. In this model T cell "recognition" of MHC and antigen consists of several independent but connected interactions of T cell surface structure with MHC molecules and antigen on antigen-presenting cells or targets.

ACKNOWLEDGMENTS

I want to thank those who collaborated with me in various stages of the experimental work: Peter Panfili, Gunther Dennert, Susan Wormsley, Risë Schwab, Janet Yamamoto, and especially Richard Dutton. I give particular thanks to Michele English for her excellent technical contributions to all phases of this project. Finally, I am indebted to those who have generously contributed cell lines and/or reagents, especially Deno Dialynas and Frank Fitch who provided the anti L3T4 and anti LFA-1 monoclonal antibodies. Supported by grants USPHS AI-08795, ACS IM-1-0, and Investigatorship from the American Heart Association.

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Immunological Reviews

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Correlation of Human Surface Parameters

L. L. LANIER
N. L. WARNE

Recognition of antigens through the surface of lymphoid cells. These antigen receptors, with the aid of sorting, have been divided into subsets. Of particular interest is the surface antigen of the cell. An antigen is as a negatively selected biochemical. It is possible to understand function by a

Several methods using monoclonal selection, can identify subsets of cells

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Immunological Rev. (1983), Vol. 74

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T Cell Subsets and the Recognition of MHC Class

SUSAN L. SWAIN

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INTRODUCTION

It is the purpose of this review to summarize our recent observations and those of other researchers on several aspects of T cell subsets as they relate to recognition of major histocompatibility complex (MHC) antigens. These observations have led us to conclude that there are two subsets of T cells which recognize respectively the two classes of MHC antigen (Class 1 and Class 2) and which can be identified by their Lyt (murine) or Leu (human) phenotype. Several features of the T cell recognition of MHC have led us to propose that there are two separate components of this recognition. In one component, T cell molecules (probably related to or identical to the Lyt or Leu molecules) interact with an invariant part(s) of either the Class 1 or Class 2 molecule which is monomorphic for that class. In the second component, the "T cell receptor" (which could either be independent or linked to the class-interaction molecule) recognizes the polymorphic determinants of the same MHC molecule. This recognition would be of the MHC alone in the dual recognition model or of MHC plus non-MHC antigen in the interaction antigen or altered self-model. The three sets of experimental observations, which will be presented below, led to the following conclusions:

- 1) The Lyt or Leu phenotype and susceptibility to blocking with appropriate antibodies of the vast majority of murine and human T cells correlates with the Class of MHC molecule recognized by those T cells.
- 2) Xenogeneic T cells respond only to allele-specific MHC determinants and are not directed to species-specific non-MHC or monomorphic MHC determinants.
- 3) T cells responding across species barriers make the same distinction of MHC Class as do T cells participating in allogeneic or syngeneic interactions.

Department of Biology, University of California at San Diego, La Jolla, California 92093, USA.

EXPERIMENTAL OBSERVATIONS

1. *Lyt and Leu phenotypes and susceptibility to MoAb blocking are associated with recognition of MHC class not with T cell function*

The experiments of Cantor and Boyse (1976) in the mouse had established that in most cases helper T cells express Lyt1 but not Lyt2 molecules while cytotoxic and some suppressor cells express Lyt2 but not Lyt1 molecules. It had also been demonstrated that helper cells recognize allogeneic or are restricted to self Class 2 MHC molecules while cytotoxic T cells recognize allogeneic or are restricted to self Class 1 molecules (for early reviews see Bach et al. 1976, Sprent 1978, Doherty et al. 1976). Thus, it was established that there is a triple correlation in which helper T cells are Lyt1^+2^- and recognize Class 2 MHC antigens while cytotoxic T cells are Lyt1^-2^+ and recognize Class 1 MHC antigens.

We showed in our earlier experiments that there can be exceptions to the correlation between T cell function and MHC class in the response to foreign MHC alleles (discussed in Dutton et al. 1978). In an analysis of these exceptions, we were able to show that the cell surface phenotype of the T cell correlates more faithfully with the class of MHC molecule recognized than with the T cell function. In bulk cultures of normal spleen cells, helper T cells that recognized Class 1 K- and D-encoded antigens were Lyt2^+ while cytotoxic T cells, both in bulk populations and in one cloned line that recognized IA, were either Lyt2 -negative (cloned line) or expressed much lower amounts of Lyt2 than CTL directed against K or D allo-antigens (Swain & Panfili 1979, Swain et al. 1979, Swain et al. 1981). We also showed that in the case where there was an unusual correlation between class recognition and function there was no restriction to the usual class of MHC (Swain 1981a).

TABLE I

Lyt 2 Expression and Susceptibility to Anti Lyt 2 Blocking is Associated with Class 1 MHC Recognition by T Cells of Several Functions

MHC Subregion Recognized	Function	Lyt 2 Expression	Anti-Lyt 2 Blocking
<i>Class 1</i>			
	Help (1°)	+++	+++
	IL2 production (2°)	+++	+++
	Cytotoxicity (1° and 2°)	+++	++
<i>Class 2</i>			
	Help (1°)	—	—
	IL2 production (2°)	—	—
	Cytotoxicity (1°)	+/-	+/-
	Cytotoxicity (2°)	+/-	+/-

Based on data from Swain and Panfili (1979), Swain (1981) and Swain, (unpublished) on the allogeneic T cell response.

Class 1 Specif
Expt. 1
 $\text{K}^b \rightarrow \text{K}^{\text{bm1}}$

Expt. 2
 $\text{K}^b \rightarrow \text{K}^{\text{mb1}}$

Expt. 3
 $\text{D}^d \rightarrow \text{D}^d$

Class 2 Specif.
Expt. 1
 $\text{I}^a \rightarrow \text{I}^k$

Expt. 2
 $\text{I}^a \rightarrow \text{I}^k$

Expt. 3
 $\text{H-2}^b \rightarrow \text{H-2}^{\text{bx d}}$

Mls-Specific
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TABLE II
Reciprocal Blocking of Allo-help by MoAbs to Lyt2 and L3T4

	T Cells	B Cells	MoAb Specificity	PFC/culture
Class 1 Specific				
Expt. 1 K ^b →K ^{bml}	—	B6.bml	—	2 (1- 2)
	B6	+	—	203 (181-227)
	+	+	L3T4	271 (228-323)
	+	+	Lyt2	24 (20- 28)
	+	+	LFA-1	<2
Expt. 2 K ^b →K ^{bml}	—	B6.bml	—	≤2
	B6	+	—	452 (381-536)
	+	+	L3T4	351 (308-399)
	+	+	Lyt2	12 (5- 28)
Expt. 3 D ^a →D ^d	—	B10.T (6R)	—	<
	B10.G	+	—	83 (70- 99)
	+	+	L3T4	60 (56- 64)
	+	+	Lyt2	9 (4- 19)
Class 2 Specific/or Whole Haplotype				
Expt. 1 I ^a →I ^k	—	B10.AQR	—	2 (1- 3)
	B10.T (6R)	+	—	435 (408-465)
	+	+	L3T4	21 (9- 52)
	+	+	Lyt2	502 (476-531)
Expt. 2 I ^a →I ^k	—	B10.AQR	—	<2
	B10.T (6R)	+	—	36 (26- 48)
	+	+	L3T4	6 (3- 9)
	+	+	Lyt2	30 (25- 36)
Expt. 3 H-2 ^b →H-2 ^{bxd}	—	BDF ₁	—	4 (2- 11)
	B6	+	—	1,056 (1,024-1,087)
	+	+	L3T4	98 (91-106)
	+	+	Lyt2	626 (556-704)
	+	+	LFA1	3 (2- 5)
Mls-Specific				
Expt. 1 Mls ^b →Mls ^a	—	D1.LP	—	149 (120-184)
	B6	+	—	614 (442-853)
	+	+	L3T4	45 (35- 57)
	+	+	Lyt2	791 (711-880)
	+	+	LFA-1	11 (7- 17)

In these experiments T-depleted B cells developed PFC in response to SRBC and were helped by allogeneic T cells that differed at either Class 1 or Class 2 loci or at Mls. The monoclonal antibodies were present throughout the 4-day culture period.

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The same correlations could be shown in experiments where Lyt2 monoclonal antibodies were used to block activity. The helper function or IL2 production of cells responding to K or D allo-antigens could be blocked by monoclonal anti-Lyt2, while cytotoxic function specific for IA or whole I region could not be blocked by the same reagent (Swain 1981b). These findings are summarized in Table I. It recently became apparent that Lyt1 is not expressed reciprocally to Lyt2 (Swain & Dutton 1981, Ledbetter et al. 1981) and does not block Class 2 specific function. Thus, at the time these initial experiments were done it was not possible to examine the blocking of T cell responses to Class 2 antigens because of the lack of an appropriate reagent to define a true reciprocal of Lyt2 (but see below).

More recently it has been shown that the same pattern of observations can be documented in humans, mainly in experiments utilizing cloned lines (Engelman et al. 1981, Krensky et al. 1982, Reinherz et al. 1981, Biddison et al. 1982, Meuer et al. 1982). In humans, the marker, Leu 1, would appear to be the equivalent of the mouse Lyt1 that it is expressed to varying degrees on all T cells. Antisera to the Lyt1 and/or Leu 1 do not generally block responses. Leu 2 appears to correspond to Lyt2 (Ledbetter et al. 1981). Leu 3, however, has a reciprocal distribution to Leu 2 and appears to define the T cell subset that recognizes or is restricted to Class 2 MHC antigen. Antibody to Leu 3 has been shown to block the response to Class 2 antigen.

An equivalent marker, L3T4, has now been found in the mouse by Dialynas

TABLE III
Reciprocal Blocking of IL2 Production by MoAbs to Lyt2 and L3T4

Primed Responders	Stimulator B10.T (6R)	Specificity of MoAb	CPM/Culture with 10% Supernatant
None	—	None	68 (60–77)
<i>Class 1 Reactive</i>			
1. B10.G	+	None	461 (434–489)
2. B10.G	+	Lyt2	112 (111–113)
3. B10.G	+	L3T4	560 (546–574)
4. B10.G	+Con A	None	971 (802–1,176)
<i>Class 2 Reactive</i>			
5. B10.AQR	+	None	830 (656–1,046)
6. B10.AQR	+	Lyt2	457 (356–586)
7. B10.AQR	+	L3T4	81 (77–86)
8. B10.AQR	+Con A	None	956 (825–1,101)

Primed T cell populations were prepared in response to stimulators allogeneic at Class 1 or Class 2 MHC. The primed T cells were re-stimulated with the appropriate stimulators in the presence or absence of monoclonal antibodies to Lyt2 or L3T4 and the 24-h IL2 production assayed on an IL2 dependent cell line as previously described (Swain et al. 1983).

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and his colleagues, and is defined by the monoclonal antibody GK1.5 (Dialynas et al. 1983). We were then able to complete our analysis in the mouse and report here that GK1.5 (anti-L3T4) can block allogeneic help and lymphokine production elicited by Class 2 but not Class 1 MHC. The results, using bulk cultures, are illustrated in Tables II and III. It can be seen that antibody to L3T4 was able to inhibit both allo-help (Table II) and the production of IL2 (Table III) to I region antigens and Mls, but not to those encoded by K and D subregions. It is of interest that monoclonal antibody to LFA 1 (an unrelated cell surface antigen) which had been previously reported to block killing to K and D (Davignon et al. 1981) showed no class recognition selectivity and blocked helper responses to both I and K/D. The impressive correlation of Lyt phenotype, blocking with Lyt antibodies and recognition of MHC subregion in the mouse is summarized in Table IV.

2. Xenogeneic T cell responses recognize only allelic specificities

Lindahl and Bach (1975, 1976) showed that the response of human T cells to mouse lymphocytes was allele-specific and that there was no response to the non-polymorphic 'mouse' MHC determinants.

We have confirmed and extended these observations in our own study of human anti-mouse mixed lymphocyte responses (Swain et al. 1983). In these studies we showed that T cells stimulated with mouse lymphocytes in a primary *in vitro* response could only be re-stimulated by MHC compatible mouse lymphocytes in a secondary response. Responses re-stimulated with whole H-2 compatible or Class 1-compatible stimulators resulted in the development of

TABLE IV
Reciprocal Blocking of Allogeneic T Cell Reactivity to Class 1 and Class 2 MHC Subregion Antigens

MHC Subregion Recognized	Function	Anti-Lyt2 Blocking	Anti-L3T4 Blocking	Anti-LFA-1 Blocking
Class 1				
	Help (1°)	+++	-	++++
	IL2 production (2°)	+++	-	++++
	Cytotoxicity	+++	-	++++*
Class 2				
	Help (1°)	-	+++	++++
	IL2 production (2°)	-	+++	++++
	Cytotoxicity (1° and 2°)	+/-	++	++++

* Data from Davignon et al. 1981. Based on data in this review and in Swain, Dialynas, Fitch and English, manuscript in preparation.

TABLE V
Specificity of Human T Cells Responsive to Mouse Stimulators

Restimulator or target genes shared with primary stimulator	Response measured (Degree)	
	IL2 production	Target lysis
Whole MHC \pm Background	+++	+++
Background	—	—
Class 1 \pm Background (K or D)	—	+++
Class 2 \pm Background	++++	—

This data is a summary of our experience with human anti-mouse responses. Details and experimental methods can be found in Swain et al. (1983). Primary cultures of 4×10^7 human PBLs and 1×10^7 mouse splenic T-depleted, mitogentreated stimulators were cultured for 7–10 days in 20 ml in flasks. Cells were recultured with mouse 2° stimulator cells from mice sharing MHC and/or background genes with those used in the primary culture. IL2 production was determined by assaying the 24-h culture supernatants of 2° cultures. Cytotoxicity was determined on Con A plus LPS blasted mouse spleen cells.

CTL activity specific for the relevant Class 1 allele, while responses re-stimulated with Class 2-compatible (to responder) splenic non-T cells generated IL2 production. These results are summarized in Table V.

Two features of these responses can be emphasized.

- 1) The response was all directed to allele-specific (polymorphic) determinants, i.e., there was no measurable response to any shared mouse MHC (monomorphic) determinants or to other non-MHC (background) determinants.
- 2) The human T cells were able to make the same functional distinction between murine Class 1 and Class 2 MHC as in the allogeneic and syngeneic response in that the appropriate T cell function was correlated with the MHC class recognized.

3. T cells which distinguish MHC class across species barriers are appropriately blocked by MoAbs to Leu antigens

As already indicated in the previous section, the response of human T cells to mouse Class 2 antigens resulted in IL2 production while CTL were generated in response to Class 1 antigens. The fact that human T cells distinguished mouse Class 1 and Class 2 structures in analogous manner to that in responses within the species was further illustrated by blocking experiments with anti-Leu 2 and anti-Leu 3 reagents. The cytotoxic response to K and D was blocked by anti-Leu 2 while the IL2 production elicited by restimulation with Class 1 was blocked by anti-Leu 3. These findings (Swain et al. 1983) are illustrated in Table VI.

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TABLE VI
Susceptibility to Anti-Leu 2a and 3a Blocking of 2° Human and Mouse Responses

Specificity of Anti-Leu Antibody	IL2 Production	% specific Cytotoxicity
	Δ CPM	
None	768	22
Leu 2a	804	8
Leu 3a	-8	26

Primary cultures of human PBLs were set up against B10.A (3R) mouse, T-depleted splenic stimulators. After 10 days cultures were re-stimulated with B10.A (3R) T-depleted spleen cells. Supernatants were harvested after 18 h to determine IL2 production and specific cytotoxicity was determined after 4 days on LPS plus Con A B10.A (3R) blasts. Further details in Swain et al. 1983.

DISCUSSION

1. Implications of experimental observations

Before going on to a broader discussion of T cell recognition, the significance of the apparent involvement of Lyt and Leu antigens in T cell recognition of MHC class will be analyzed.

The correlation of the expression of the Lyt2 antigen with recognition of Class 1 MHC is very impressive when bulk populations of normal or short term *in vitro* primed T cells from mouse spleen are used in the functional assays used in our experiments. These include unprimed and *in vitro* primed allo-help and IL2 production (Swain 1981b) and *in vivo* primed cytotoxic responses (Swain 1981b). In contrast, published results in unprimed cytotoxic responses in the mouse are somewhat less consistent, for instance: while Vidovic et al. (1981) found most Class 2-specific killers came from Lyt2⁻ precursors, Miller & Stutman (1982) found anti-Lyt2 blocking of both Class 1- and Class 2-directed cytotoxic T cells. In our experiments, Class 2-specific precursors of cytotoxic effectors expressed less Lyt2 than those directed to Class 1, but a certain proportion were, nonetheless, removed by anti-Lyt2 and C treatment. Furthermore, among hybridomas and cytotoxic clones are found examples of Class 1-specific cytotoxic effectors which do not appear to express Lyt2 (MacDonald et al. 1982) and which are not always blocked by anti-Lyt2 reagents (MacDonald et al. 1982). There are several possible explanations for these deviations from the Lyt-MHC subregion rule.

The first explanation, only applicable to the bulk CTL responses, is that some T cells in putative I region only different cultures, may really be specific for non-K or -D Class 2 antigens including L, R, Qa and possibly others. This is not unlikely in bulk cultures and indeed among *cloned* I-specific (mouse) and DR-

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specific (human) lines or clones where the specificity has been determined, a very good correlation has been found, i.e., an absence of Lyt2 and Leu 2 markers and presence of L3T4 and Leu 3 (Swain et al. 1981, Dialynas et al. 1983, Krensky et al. 1982, Meuer et al. 1982).

However, this explanation does not deal with T cell clones which can be shown to be specific for Class 1 and yet lack and/or are not blocked by, anti Lyt2. The exceptions in these cases put important constraints on the possible roles for Lyt2. Long term lines and clones, however, may not always accurately reflect normal phenotypes. Nonetheless, it is clear that Lyt2 molecules are *not always* involved in T cell recognition of Class 1 MHC.

In order to interpret these results, several more facets of blocking with monoclonal and polyclonal antibodies are relevant.

(1) Very few reagents specific for T cell surface markers have been found which are able to block T cell function despite extensive attempts to find such reagents (reviewed in Immunol. Rev. 68). The first of these, and one of the two most commonly found, are reagents to Lyt2 which were first found to block cytotoxic function by Shinohara and Sachs (1979) and Nakayama et al. (1979). Since those initial reports, a wide variety of studies have confirmed and extended these observations (reviewed in Immunol. Rev. 68). The only other commonly found antibodies which block function are those against the high molecular weight antigens termed LFA-1 (Davignon et al. 1981, Pierres et al. 1982) which are large proteins (94-108K) expressed on all subsets of T cells (as well as B cells). Antibody to LFA-1 blocks multiple T cell functions (all tested) regardless of specificity (Davignon et al. 1981, Pierres et al. 1982, and results in this report).

(2) Anti-Lyt2 reagents appear to block cytotoxicity (both induction and effector function) at the very early phase of precursor/effector interaction with the target (Fan et al. 1980, MacDonald et al. 1982, Goldstein et al. 1982, Hollander 1982, Springer et al. 1982). Furthermore, lectins such as Con A overcome both the target specificity of killing and blocking with anti Lyt2. Allo-help and IL2 production are also blocked at the induction stage (Swain 1981b) and Con A also overcomes both the specificity and blocking (Swain 1981b). In all cases the T cell is clearly the target. Both of these facets suggest anti-Lyt2 blocks recognition.

(3) Finally, molecules of similar structure are found in many species (mouse, human, rat) and in all cases antisera and monoclonal antibodies to these structures have been found which block function.

Taken together, the above three sets of observations make a persuasive, though somewhat circumstantial case that the molecules in question (Lyt2 and L3T4; Leu 2, Leu 3) play an important role in T cell response to MHC antigen. The most likely role from these and our own observations would be in recognition of MHC class.

The negative data suggests that whatever the role of Lyt2, it is not always

obligatory. C and Leu-enc minants. W interaction c which partici nominal ant lectin-facilita tion. In fact, anti-Lyt2 fai block a cross involvement the same gro from periton derived from

2. Speculation

The experim discussion of above but a r They are list

(1) Appro: foreign MHC the earliest a majority of a feature was r that all T cel et al. 1973, 2 explanations specificities repertoire wa a single MH.

(2) The T molecule. Th antigen (Zinl might be arg was due to induction to polymorphic several othe explanation extremely co

obligatory. One way to reconcile these two phenomena is to suggest that the Ly- and Leu-encoded molecules interact with monomorphic class-specific determinants. We suggest that this interaction contributes to or facilitates the interaction of the clonally distributed antigen-specific part of the receptor(s) which participate(s) in recognition of polymorphic determinants on MHC and of nominal antigen. It is thus possible that high affinity specific receptors or lectin-facilitated binding would not require the facilitatory class-specific interaction. In fact, data from MacDonald et al. (1982) includes one instance in which anti-Lyt2 failed to block the original antigen-induced cytotoxic activity but did block a cross-reaction of that same clone consistent with a requirement for Lyt2 involvement only when a, presumably, lower affinity interaction occurs. Also the same group previously demonstrated that primed cytotoxic effectors derived from peritoneal exudate were less susceptible to anti-Lyt2 blocking than those derived from spleen (MacDonald et al. 1981).

2. Speculations on T cell recognition

The experimental observations that we would like to consider in further discussion of how T cells recognize MHC include not only those summarized above but a number of earlier basic features of the T cell interaction with MHC. They are listed and discussed in sequence.

(1) Approximately 1% of the T cell population is activated in response to a foreign MHC allele. The exact figure is not important, but it has been clear from the earliest experiments (Dutton 1965, Nisbet et al. 1969) that perhaps the majority of all T cells must be reactive to one or another foreign allele. This feature was neither explained or rendered less remarkable when it was revealed that all T cell responses to non-MHC antigens are restricted to self-MHC (Katz et al. 1973, Zinkernagel & Doherty 1975). There would appear to be only two explanations: either that the recognition structures consisted of a limited set of specificities restricted to the recognition of allogeneic MHC or that the repertoire was "open" (and large) but there is a "bias" towards MHC that allows a single MHC allele antigen to capture a large portion of the T cell repertoire.

(2) The T cell recognition of MHC is limited to the polymorphic parts of the molecule. This is seen both in the self-MHC restricted response to non-MHC antigen (Zinkernagel & Doherty 1979) and in the response to allogeneic MHC. It might be argued that the lack of a response to the invariant part of the molecule was due to the elimination of such reactivity by a mechanism of tolerance-induction to self-components. The finding of a similar preoccupation with polymorphic determinants in xenogeneic responses discussed above and seen in several other xenogeneic situations, however, would seem to rule out an explanation based on self-tolerance, unless the non-polymorphic regions were extremely conserved between species, which, from the data available, does not

seem to be the case. Again there would seem to be a constraint either on the repertoire of receptor structures itself and/or upon the way it functions; e.g., some sort of obligatory interaction with MHC polymorphic regions.

(3) T cell clones specific for non-MHC antigens restricted to self-MHC (self+X) often cross-react with a foreign MHC allele (originally described by Sredni & Schwartz 1981). The frequency of such reactivity in some cases is so high as to suggest that perhaps all T cells react with one or another of the species' polymorphic structures of MHC. Further interpretation of this is heavily weighted by whether one considers a two-receptor or one-receptor model for T cell recognition. Several pieces of information presented in this review seem relevant here.

(4) T cells that recognize Class 1 MHC antigens carry different cell-surface molecules from those that recognize Class 2 MHC antigens. (5) This recognition operates across species barriers and (6) the consequences of recognition can be blocked by the presence of antibody to the appropriate cell-surface marker. We consider that the most likely conclusions to be drawn from this are: first, that the recognition of MHC class is carried out independently from the recognition of MHC polymorphic determinants (and the non-MHC antigen); and second, that the relevant cell surface structures are in some way associated with this recognition as outlined earlier.

3. A model for the T cell receptor

We have, therefore, proposed a model (Swain et al. 1983) in which the recognition of MHC class is separated from the recognition of MHC poly-

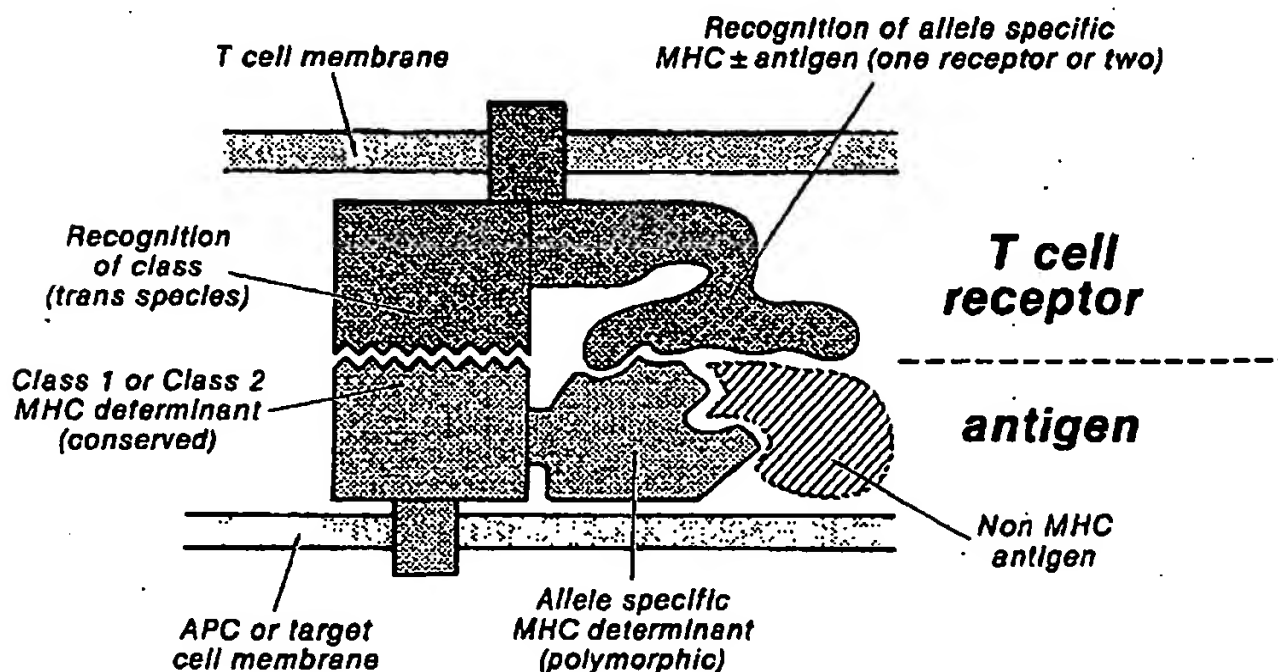


Figure 1. Hypothesis for recognition of MHC class and polymorphic determinants by T cells. This model was previously published in *J. Exp. Med.* 157, 720.

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morphic determinants and non-MHC antigen (see Figure 1). We further suggest that the recognition of the class-specific part of the MHC molecule 'positions' the receptor so that it can only interact with area where the polymorphic variations occur. The concept conveyed by the model can be drawn in many ways and the version presented here is merely one presentation of the principles involved. We have drawn the separate components of the model joined together with a single attachment to the membrane. The two components could be independently attached and need not be associated with one another before the interaction occurs or even then. In its essence, this model is not affected by whether MHC and non-MHC are recognized by one or two recognition events.

SUMMARY

We have presented and/or briefly reviewed data which indicates that there are two T cell subsets which interact respectively with the two Classes (1 and 2) of MHC antigen and which can be identified by the Ly (mouse) or Leu (human) molecules that they express. This correlation, and the large body of (largely) circumstantial but still quite convincing data, suggests that these Ly and Leu molecules play a very important role in T cell responses by actually interacting with monomorphic MHC class specific determinants. We suggest that this interaction facilitates and possibly helps direct the binding of the T cell receptor to polymorphic MHC determinants and antigen. In this model T cell "recognition" of MHC and antigen consists of several independent but connected interactions of T cell surface structure with MHC molecules and antigen on antigen-presenting cells or targets.

ACKNOWLEDGMENTS

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Immunological Reviews

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Correlation of Human Surface Parameters

L. L. LANIER
N. L. WARNE

Recognition of antigens through the dendritic lymphoid cell surface, these antigens with the analysis of sorting, has led to subsets. Of particular surface antigens of the cell. All antigens are as negatively selected biochemically possible to use function by a. Several methods using monoclonal selection, co-selection of subsets of cells

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MONOCLONAL ANTIBODY TO L3T4 BLOCKS THE FUNCTION OF T CELLS SPECIFIC FOR CLASS 2 MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS¹

SUSAN L. SWAIN,* DENO P. DIALYNAS,† FRANK W. FITCH,† AND MICHELE ENGLISH*

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The ability of antibody to Lyt-2 and to the newly described L3T4 antigen to block the functions of helper T cells with specificity for allogeneic class 1 or class 2 MHC subregion antigens was determined. Anti-Lyt-2 blocked both allohelp delivered by unprimed T cells and IL 2 production by primed T cells when the response was directed to class 1 MHC antigens, but had no effect when the response was directed to class 2 MHC or MIs antigens. Anti-L3T4 had reciprocal activity. A control reagent, anti-LFA-1, blocked all responses tested regardless of specificity. This and other reports provide strong evidence that L3T4 is the murine equivalent of the human T cell markers Leu 3/OKT4. The ability of antibodies directed against Lyt-2 and L3T4 to block T cell function in a fashion determined by the class of MHC antigen recognized by the T cell further supports the hypotheses that Lyt-2 and L3T4 molecule in the mouse and Leu-2 and Leu-3 molecules in the human are involved in the interaction of the T cell with class-specific determinants on MHC molecules.

It has become clear in the past decade that most, if not all, helper and cytotoxic T cells must recognize and interact with major histocompatibility complex (MHC)² molecules to function. This requirement is reflected in non-MHC antigen-specific responses by the phenomenon of MHC restriction and in nonhomologous responses in the very large T cell response to allogeneic and xenogeneic MHC antigens.

There are several striking features both of the T cell response to MHC antigens and of the MHC antigens themselves. First there are two classes of MHC molecules, which are found in all species studied and are of quite different structure; these are termed class 1 and class 2 antigens (reviewed in Reference 1). The class 1 molecules, the original transplantation antigens, consist of a single transmembrane MHC-encoded polymorphic chain of 40,000 to 45,000 m.w. associated with non-MHC-encoded β_2 -microglobulin chain. In the mouse, the class

1 molecules include the well-studied K and D-encoded molecules. The recognition of these class 1 molecules is generally correlated with T cells that have cytotoxic function. The class 2 molecules consist of two MHC-encoded transmembrane chains of approximately 34,000 and 28,000 m.w., termed α and β -chains. These correspond to the I region molecules of the mouse, which include the I-A and I-E region molecules. The recognition of class 2 molecules is generally correlated with T cells that have helper or inducer function (1-4). Thus there is a functional as well as chemical distinction between class 1 and class 2 MHC molecules in their role as antigens recognized by T cells.

Subsets of T cells have also been identified by their expression of a series of surface markers termed Lyt in the mouse and Leu or OKT in the human. It was appreciated quite early that expression of these markers on T cells showed a good association with function. Thus, in the mouse, the subset of T cells expressing Lyt-1, but not expressing Lyt-2, was found to contain the major fraction of helper activity, whereas that subset expressing Lyt-2 contained most T cells capable of cytotoxic function (5). This pattern was later reproduced in studies on human T cells, which also yielded new and important insights. Human marker(s) were found that corresponded to Lyt-2 called Leu 2 or OKT5/8 (6-8). By this time it had become appreciated that Lyt-1 in the mouse was not reciprocally expressed with Lyt-2; many functional T cells had the Lyt-1⁺2⁺ phenotype (5, 8, 9), and all T cells expressed some Lyt-1 (9-11). In the human, however, a marker termed Leu-3 or OKT4 was found that did show good reciprocal expression with Leu-2 and did not have the molecular characteristics of Lyt-1 (7, 8). An exciting new dimension was added to the study of these molecules with the reports of Shinohara and Sachs (12) and Nakayama et al. (13) that antibody to Lyt-2 had the unique ability to block most cytotoxic function. This conclusion was quickly confirmed by a number of other workers with monoclonal antibodies (MoAb) (14-16). Studies on the human T cell subsets confirmed this pattern because MoAb to Leu-2 blocked most allogeneic cytotoxicity (17, 18). Most interestingly, in the human, antibody to Leu-3 was able to block proliferation, a response usually associated with helper function (18). Recently we extended this observation to show that anti-Leu-2 blocks cytotoxic function and anti-Leu-3 blocks IL 2 production when human T cells respond to xenogeneic mouse stimulators (19). These studies and several of the details of the blocking, which include the facts that blocking occurs during early events of cell interactions but not during subsequent steps (20, 21), and that the lectins such as concan-

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² Abbreviations: MHC, major histocompatibility complex; MoAb, monoclonal antibody.

avalin A (Con A) and phytohemagglutinin can overcome blocking (14, 22), fueled speculation that Lyt and Leu molecules were active in T cell recognition.

The original descriptions of these Lyt and Leu molecules tended to suggest that they were markers associated with T cell function. Although there is a strong association between phenotype and function, there is also a correlation between Lyt/Leu phenotype and the class of MHC molecule to which the T cells are directed. We first raised the possibility that Lyt phenotype showed a better correlation with the class of MHC molecule recognized by the T cell than with T cell function in studies involving allogeneic responses to different MHC subregions (9, 16, 23). It was possible in the allogeneic situation to study the Lyt phenotype of allogeneic helpers specific for either class 1 or class 2 MHC antigens and of cytotoxic effectors with similar subregion specificities. In all of these situations, the expression of Lyt-2 was correlated with class 1 MHC recognition rather than with function (16, 23). These studies were later extended to include a T cell line with cytotoxic activity that was specific for class 2 (24). Finally we tested the ability of MoAb to Lyt-2 to block these subregion-specific responses, and again the blocking was correlated with recognition of class 1 molecules rather than with function (16). This set of phenomena was incomplete, however, because no reciprocal molecule associated with class 2 MHC recognition was known and antibodies directed to Lyt-1 did not block T cell responses (14, 16).

Experiments in the human systems designed to study the association of Leu expression as well as the ability of MoAb to Leu molecules to block function again confirmed and extended the phenomena in the murine system. It was found in several independent studies of human T cell lines that Leu-2 expression and the ability of anti-Leu-2 to block was strongly associated with recognition of class 1 HLA molecules, whereas Leu-3 expression and the ability of anti-Leu-3 to block was strongly associated with recognition of class 2 HLA molecules (25-27).

The association of Lyt and Leu expression with MHC subregion recognition and the unusual ability of MoAb to these structures to block T cell function are strong evidence for some involvement of these molecules in T cell-MHC interaction, and further suggest that these molecules might play a role in discriminating class 1 from class 2 MHC molecules.

Recently Dialynas and colleagues (26, 28) developed a MoAb with specificity for murine T cell surface molecules that are reciprocally expressed with Lyt-2 and have the same gross biochemical characteristics in electrophoretic analysis as Leu-3 or OKT4 molecules in the human, with an apparent m.w. of 52,000. We report here that this anti-L3T4 reagent blocks primary and secondary helper T cell responses to class 2 molecules but not to class 1 molecules. These data and data (29, 30) obtained with hybridomas and cell lines provide further evidence that L3T4 is the mouse equivalent of human Leu-3 and OKT4 and gives added weight to the hypothesis that Lyt-2, L3T4, and Leu-2, Leu-3 molecules play an important role in T cell interaction with MHC molecules.

MATERIALS AND METHODS

Mice. All inbred mice were bred in our colony at the University of California, San Diego. Original breeding pairs were obtained from

the Jackson Laboratory, Bar Harbor, ME, and from Dr. Donald Shreffler (Washington University School of Medicine, St. Louis, MO).

Determination of allohelp. Allohelp is operationally defined as the striking ability of mitomycin-treated T cells to interact with allogeneic T-depleted B cells to cause them to become antibody-forming cells in *in vitro* cultures. Mitomycin C-treated T cells syngeneic to B cells give virtually no help. The cultures are constructed as reported previously (23). Briefly, T cells are obtained by nylon column passage and are mitomycin C-treated ($25 \mu\text{g}/5 \times 10^7$ T cells). B cells are obtained by treating spleen cells with MoAb to Thy-1.2 (F7D5) plus C. T cells are titrated (5×10^5 to 1×10^6) into 0.1-ml cultures containing 6×10^5 B cells and 0.1% v/v selected sheep erythrocytes (SRBC, Colorado Serums, Denver, CO). Direct (IgM) plaque-forming cells (PFC) to SRBC are determined after 4 days of culture. Geometric means (\pm standard errors) of triplicate cultures are determined. All cells are cultured in RPMI 1640 supplemented with penicillin, streptomycin, and glutamine with 5% selected fetal bovine serum.

In vitro priming. Priming of T cells to MHC subregion differences is accomplished *in vitro* as described (31). Briefly, 2.5×10^7 spleen cells of the responding strain are cultured with an equal number of mitomycin C-treated cells of the stimulating strain in a total volume of 20 ml. After 10 to 14 days, cultures are harvested and restimulated. Such priming typically results in at least a 10- to 20-fold increase in the specific response and loss of third party responses (31).

Determination of IL 2 production. The cultures described above containing primed T cells are cultured at $2.5 \times 10^5/\text{ml}$ with $5 \times 10^6/\text{ml}$ of anti-Thy-1.2 (F7D5) plus C, mitomycin C-treated stimulators, and the supernatants are collected after 16 to 20 hr. The presence of IL 2 is detected by adding the supernatants at concentrations from 50 to 1% to triplicate cultures of 5×10^5 IL 2-dependent T cells from an NK line, as described previously (32). Proliferation of cells was determined by incorporation of ^{125}I iodouridine deoxyribose added during the last 24 hr of a 48-hr culture (32). Results are expressed as the mean cpm of triplicate cultures.

Blocking with MoAb. Culture supernatants from *in vitro* cultured hybridomas producing antibody to Lyt-2, L3T4, and LFA-1 (details below) were added at the initiation of the cultures of allohelpers and B cells or at the initiation of cultures for the production of IL 2. Concentrations of such antibodies ranged from 20 to 2% for the allohelp cultures and from 5 to 1% for IL 2 production.

MoAb. Anti-Thy-1.2 (F7D5) was a kind gift of Phil Lake. The culture supernatants containing anti-Lyt-2 were obtained from the rat hybridoma 53.6.72, which was obtained from the Salk Institute Cell Culture Facility and was donated by Drs. Ledbetter and Herzenberg. The culture supernatants containing anti-L3T4 were obtained from the GK1.5 hybridoma (30) and the culture supernatants containing anti-LFA-1 were from the FD4418 hybridoma (22) of Dr. Dialynas.

RESULTS

Anti-Lyt-2 and anti-L3T4 reciprocally block allogeneic help specific for class 1 and class 2 MHC antigens, respectively. The effect of anti-Lyt-2 and anti-L3T4 on allogeneic help specific for class 1 or class 2 MHC antigens was determined. T cells were taken from spleens of inbred mice and titrated into cultures of T-depleted spleen cells from allogeneic inbred mice as a source of B cells. Inbred strains were chosen that differed only in H-2K or H-2D (class 1) or at the I region (class 2). Anti-Lyt-2, anti-L3T4, or anti-LFA-1 were included at various concentrations in parallel cultures. In all cases, the allohelp was assessed by the number of PFC per culture that developed in 4 days to SRBC as antigen. The results of a representative example of such an experiment are illustrated in Figure 1. In Figure 1a, B6 T cells were titrated against B cells of the B6.bm1 strain, which express a mutant form of the K^b molecule. This strain combination gives a very strong allogeneic helper response even though the difference in MHC is restricted to class 1 antigens alone. Culture supernatant containing MoAb to Lyt-2 completely abrogated this response. In contrast, culture supernatants containing MoAb to L3T4 had no significant effect on allogeneic help. Antibody to LFA-1

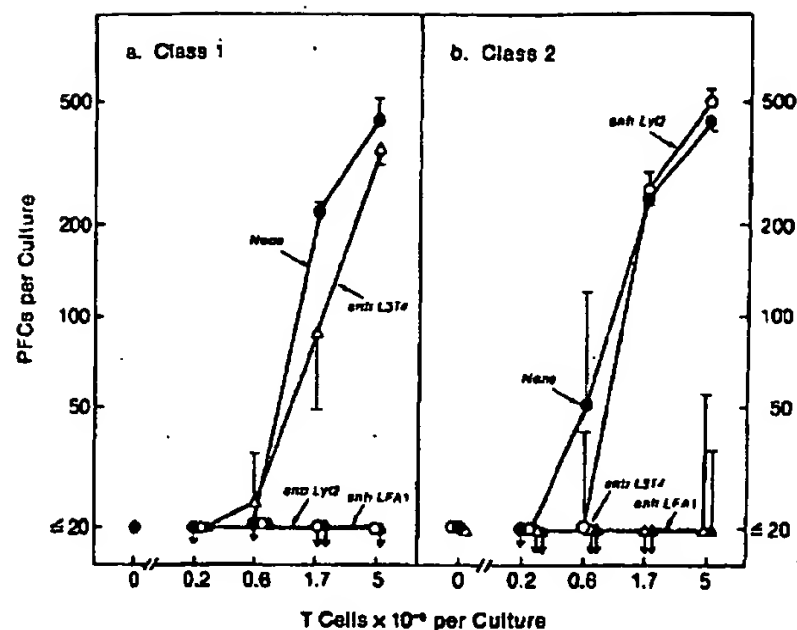


Figure 1. Blocking of MHC specific allohelp. a, mitomycin-treated B6 T cells were titrated at numbers indicated into triplicate cultures containing 6×10^6 B cells (T-depleted spleen) from B6.bm1 mice. Cultures were set up with no addition (●); with 2% culture supernatant from the GK1.5 line (anti-L3T4) (Δ); with 2% culture supernatant from the 53.6.72 line (anti-Lyt-2) (▲); or with 2% culture supernatant from the FD4418 line (anti-LFA-1) (○). Mean PFC per culture \pm SEM are indicated. b, mitomycin-treated B10.AQR T cells were titrated into triplicate cultures of B10.T(6R) B cells as in a. The same additions were made as in a.

TABLE I
Reciprocal blocking of allohelp by MoAb to Lyt-2 and L3T4

	T Cells	B Cells	MoAb Specificity	PFC/Culture
Class 1-specific				
Expt. 1	-	B6.bm1	-	2 (1-2)
	B6	+	-	203 (181-227)
	+	+	L3T4	271 (228-323)
	+	+	Lyt-2	24 (20-28)
	+	+	LFA-1	<2
Expt. 2	-	B10.T(6R)	-	<2
	B10.G	+	-	83 (70-99)
	+	+	L3T4	60 (56-64)
	+	+	Lyt-2	9 (4-19)
Class 2 specific or whole haplotype				
Expt. 1	-	B10.AQR	-	<2
	B10.T(6R)	+	-	38 (26-48)
	+	+	L3T4	6 (3-9)
	+	+	Lyt-2	30 (25-38)
Expt. 2	-	BDF ₁	-	4 (2-11)
	B6	+	-	1056 (1024-1087)
	+	+	L3T4	98 (91-106)
	+	+	Lyt-2	626 (556-704)
	+	+	LFA-1	3 (2-5)
Mis-specific				
Expt. 1	-	D1.LP	-	149 (120-184)
	B6	+	-	614 (442-853)
	+	+	L3T4	45 (35-57)
	+	+	Lyt-2	791 (711-880)
	+	+	LFA-1	11 (7-17)

was also very effective in preventing a PFC response. In Figure 1b, B10.T(6R) T cells were titrated against B cells from B10.AQR mice. These strains differ over the entire I region. Again a very strong allogeneic helper response was seen. In this case, however, the same preparations of MoAb had strikingly different effects. MoAb to Lyt-2 had no effect on the response, whereas MoAb to L3T4 completely blocked responsiveness. MoAb to LFA-1 also completely blocked the allogeneic help. Similar results were seen in three other experiments with the same strain combination. Results of some of these experiments are included in Table I.

Several more comments can be made. First, when other recombinant inbred strains of mice were used as T and B cell donors differing only at class 1 MHC, weaker

allogeneic help was seen (see Table I) as reported previously (23). However, the pattern of anti-Lyt-2 and not anti-L3T4 blocking was the same regardless of whether the response was a strong one (reflecting a high frequency of T cells specific for the K^b mutant (33)) or a weaker one (reflecting the generally lower frequency of class 1-specific allohelpers). As expected, the response to a whole MHC region was susceptible to anti-L3T4 and was largely resistant to anti-Lyt-2 (Table I). Although a 40% lower response was seen in the cultures with anti-Lyt-2, this degree of blocking is not reproducible over a large number of experiments (not shown, but note previous results especially Reference 23).

Ability of anti-L3T4 to block class 2 but not class 1-specific allohelp. Table II shows a titration of anti-L3T4 antibody into a response against class 2 MHC (B10.T(6R) T cells with B10.AQR B cells) and against class 1 MHC (B10.G to B10.T(6R)). It is clear that MoAb to L3T4 showed specific blocking of T cells directed to class 2 MHC over a broad range of doses. A slight inhibition of class 1 responses was seen with the addition of L3T4 supernatants, but this sort of inhibition may reflect variation among triplicate cultures (which can be as much as twofold), and did not appear to increase with increasingly larger doses of L3T4, suggesting it is nonspecific.

Anti-Lyt-2 and anti-L3T4 reciprocally block IL 2 production by T cells primed and restimulated in vitro with class 1 and class 2 differences, respectively. T cells were primed in vitro to either class 1 or class 2 MHC subregion differences. Such T cells showed a much en-

TABLE II
Ability of anti-L3T4 to block class 2, but not class 1, specific allohelp

B Cells	T Cells	Addition	PFC/Culture
B10.AQR	B10.T(6R)	None	1236 (170-1884)
		Anti-L3T4, 20%	14 (3-54)
		10%	28 (17-48)
		5%	15 (9-24)
		2%	46 (20-106)
B10.G	B10.T(6R)	None	549 (427-705)
		Anti-L3T4, 20%	355 (315-400)
		10%	331 (291-378)
		5%	185 (160-215)
		2%	303 (285-320)

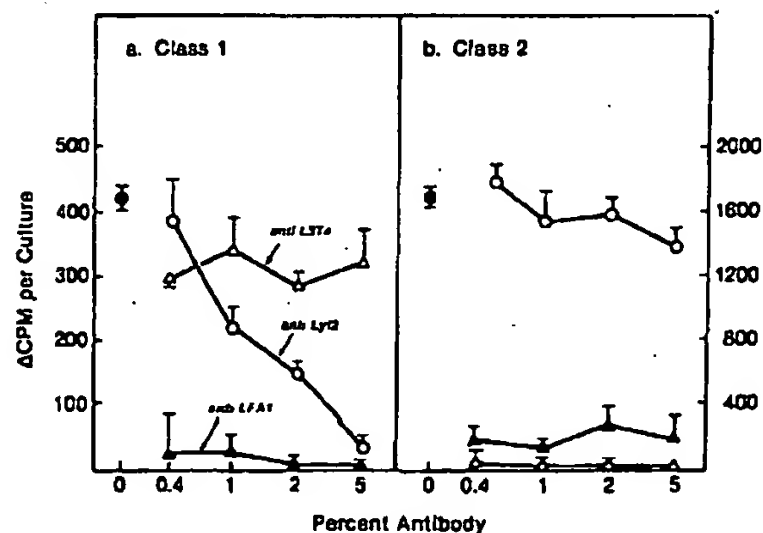


Figure 2. Blocking of secondary class 1 or 2 MHC-specific responses. a, in vitro primed B10.T(6R) T cells were restimulated in vitro with B10.G B cells. Cultures were incubated with indicated doses of supernatants from GK1.5 (anti-L3T4) (Δ); 53.6.72 (anti-Lyt-2) (○); and FD4418 (anti-LFA-1) (▲). Culture supernatants were collected after 18 hr and their content of IL 2 was assayed by the ability of 17% of supernatant to cause the proliferation (Δ cpm per culture) of an IL 2-dependent line. b, conditions were identical to a except that B10.T(6R) T cells were primed and restimulated with B10.AQR B cells.

hanced response to the priming MHC subregion difference and have lost most responsiveness to third party MHC antigens as detected both by their ability to give allohelp and by their production of the lymphokine IL 2 in response to stimulation to the priming alloantigen (31). MoAb to Lyt-2 and L3T4 were included during the restimulation with T-depleted stimulators of the priming haplotype, and supernatants were collected after 18 to 24 hr and their IL 2 content was determined. It was previously shown that the presence of these MoAb did not affect the IL 2 assay (16). The results of a representative experiment are illustrated by Figure 2. The supernatants were all titrated in the IL 2 assay, but only the proliferation of the NK targets at 17% added supernatants is shown. Similar effects were seen at 50 and 6%. Production of IL 2 in response to class 1 MHC antigen (B10.T(6R) against B10.G) in this case was blocked by MoAb to Lyt-2 but not to L3T4. In contrast, MoAb to Lyt-2 had no effect on the IL 2 production of T cells responding to class 2, whereas L3T4 was very effective at blocking in this situation. MoAb to LFA-1 blocked IL 2 production effectively in both situations. This result was found three times with these strain combinations.

DISCUSSION

The results in this report show clearly that MoAb to L3T4 and to Lyt-2 reciprocally block allohelp and lymphokine production of normal bulk cultures of T cells specific for class 2 and class 1 MHC antigens, respectively. Previously, MoAb to L3T4 was reported to interact with a T cell surface molecule that is expressed on normal T cells and in most T cell lines and hybridomas reciprocally with Lyt-2 (28-30). The molecule precipitated by the anti-L3T4 has an apparent m.w. of 52,000 and behaves similarly to Leu-3/OKT4 on polyacrylamide gel electrophoresis (28). In addition, anti-L3T4 was found to block the class 2 MHC-specific cytolytic line A15-1.17 (28, 30, 34). Thus there is good evidence that L3T4 is the murine homologue of Leu-3/OKT4, and furthermore, that the murine and human T cell surface markers might be involved in a similar fashion in interaction with class 2 MHC molecules.

The results in this paper extend this picture to bulk populations of normal T cells. With the previously reported results indicating Lyt-2 blocking of class 1 directed response regardless of T cell function, and that human T cell lines specific for class 1 expressed and were blocked by anti-Leu-2, while lines specific for class 2 expressed and were blocked by anti-Leu-3, a striking pattern emerges that is similar in mouse and man. In both species there are T cell surface molecules of similar structure strongly associated in a class-specific manner with recognition of MHC antigens. In both cases, some MoAb to these structures have the unique property of blocking the interaction of T cells with antigen-presenting cells or target cells at an early stage, which correlates temporally with recognition events.

Furthermore, as we reported previously, the xenogeneic interactions that occur when human T cells recognize mouse MHC molecules are appropriately blocked by MoAb to Leu-2 and Leu-3 structures on such human T cells (19).

In the studies in this report, the MoAb to LFA-1 consistently blocked all T cell responses, regardless of spec-

ificity. This pattern of blocking is consistent with those reported for anti-LFA-1 by Springer and colleagues (35) and other investigators (36) (D. W. Lancki et al., manuscript in preparation) and in marked contrast to the reciprocal blocking of anti-Lyt-2 and anti-L3T4, which depended on whether T cells were responding to class 1 or class 2. It is important to note that antibodies to LFA-1 and the Lyt and Leu families of molecules are the only antibodies to T cell surface markers that have been found consistently to block function except for the recent reports of clone-specific MoAb, which appear to be directed to the antigen-specific portion(s) of the cell receptor (37, 38), and antibodies to the human Leu-4/OKT3 molecule, which appear to be associated with the clonotypic receptor (41).

The impressive nature of the blocking we report in bulk cultures of T cells indicates that the vast majority of T cells taking part in the allogeneic help and lymphokine production *in situ* are susceptible to the blocking effects of the Lyt/Leu-specific MoAb, and imply that these structures or something physically associated with them are required for the interaction of the T cell with MHC molecules.

In the case of primed T cells and T cell clones, a number of investigators have described instances in which the appropriate MoAb has failed to block function, even though T cells of appropriate specificity were involved (21, 39). These observations put important constraints on the interpretation of the findings outlined above. They suggest that the interaction of Lyt/Leu molecules with MHC class-specific determinants is not always obligatory. In many cases these failures of blocking could be ascribed to interactions between T cell receptors and antigen, being of sufficient affinity to overcome blocking, as suggested by MacDonald and colleagues (21). Certainly there is considerable heterogeneity among T cell clones with respect to their susceptibility to blocking with anti-Lyt-2. MacDonald et al., however, have also presented evidence that certain T cell clones specific for class 1 molecules have no detectable expression of Lyt-2 (21). Because of the virtually complete suppression of normal bulk responses seen here and in our previous studies (16), it seems that these T cells must be rare in unprimed populations. We think the most feasible way to reconcile these findings is to postulate that Lyt-2 and L3T4 molecules in the mouse and Leu-2 and Leu-3 molecules in the human do interact with MHC molecules of class 1 and class 2, but that this interaction is not directly required for T cell triggering; instead it increases the relative avidity of the binding of the antigen-specific T cell receptor with specific antigen, including polymorphic "restricting" determinants on MHC. An analysis of spontaneously arising L3T4 loss variants of cloned class 2 MHC-reactive T cell hybridomas is consistent with the model (P. Marrack et al., manuscript in preparation). Similar concepts have been proposed in other reports by us (19, 40) and by MacDonald et al. (21), Reinherz et al. (41), and Biddison et al. (27).

The alternative explanation, made increasingly unlikely with the large amount of recent data showing a similar pattern of association of Lyt and Leu with recognition of MHC class in diverse systems, is that these T cell surface markers are not involved in interaction themselves but are "spatially" associated with structures re-

quired in T cell function, or somehow give negative signals to the cell. Although such possibilities should not be dismissed, we believe that the specificity of these molecules for either class 1 or class 2 directed interactions, their impressive evolutionary conservation revealed by the striking similarities of the murine and human systems, and the relative uniqueness of cell surface markers against which blocking antibodies can be raised, as well as the mechanistic data that indicate blocking occurs at the level of T cell interaction with other cells, provide a large amount of circumstantial evidence that the Lyt and Leu molecules are directly involved in interaction with MHC antigens.

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MONOCLONAL ANTIBODY TO L3T4 BLOCKS THE FUNCTION OF T CELLS SPECIFIC FOR CLASS 2 MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS¹

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The ability of antibody to Lyt-2 and to the newly described L3T4 antigen to block the functions of helper T cells with specificity for allogeneic class 1 or class 2 MHC subregion antigens was determined. Anti-Lyt-2 blocked both allohelp delivered by unprimed T cells and IL 2 production by primed T cells when the response was directed to class 1 MHC antigens, but had no effect when the response was directed to class 2 MHC or Mls antigens. Anti-L3T4 had reciprocal activity. A control reagent, anti-LFA-1, blocked all responses tested regardless of specificity. This and other reports provide strong evidence that L3T4 is the murine equivalent of the human T cell markers Leu 3/OKT4. The ability of antibodies directed against Lyt-2 and L3T4 to block T cell function in a fashion determined by the class of MHC antigen recognized by the T cell further supports the hypotheses that Lyt-2 and L3T4 molecule in the mouse and Leu-2 and Leu-3 molecules in the human are involved in the interaction of the T cell with class-specific determinants on MHC molecules.

It has become clear in the past decade that most, if not all, helper and cytotoxic T cells must recognize and interact with major histocompatibility complex (MHC)² molecules to function. This requirement is reflected in non-MHC antigen-specific responses by the phenomenon of MHC restriction and in nonhomologous responses in the very large T cell response to allogeneic and xenogeneic MHC antigens.

There are several striking features both of the T cell response to MHC antigens and of the MHC antigens themselves. First there are two classes of MHC molecules, which are found in all species studied and are of quite different structure; these are termed class 1 and class 2 antigens (reviewed in Reference 1). The class 1 molecules, the original transplantation antigens, consist of a single transmembrane MHC-encoded polymorphic chain of 40,000 to 45,000 m.w. associated with non-MHC-encoded β_2 -microglobulin chain. In the mouse, the class

1 molecules include the well-studied K and D-encoded molecules. The recognition of these class 1 molecules is generally correlated with T cells that have cytotoxic function. The class 2 molecules consist of two MHC-encoded transmembrane chains of approximately 34,000 and 28,000 m.w., termed α and β -chains. These correspond to the I region molecules of the mouse, which include the I-A and I-E region molecules. The recognition of class 2 molecules is generally correlated with T cells that have helper or inducer function (1-4). Thus there is a functional as well as chemical distinction between class 1 and class 2 MHC molecules in their role as antigens recognized by T cells.

Subsets of T cells have also been identified by their expression of a series of surface markers termed Lyt in the mouse and Leu or OKT in the human. It was appreciated quite early that expression of these markers on T cells showed a good association with function. Thus, in the mouse, the subset of T cells expressing Lyt-1, but not expressing Lyt-2, was found to contain the major fraction of helper activity, whereas that subset expressing Lyt-2 contained most T cells capable of cytotoxic function (5). This pattern was later reproduced in studies on human T cells, which also yielded new and important insights. Human marker(s) were found that corresponded to Lyt-2 called Leu 2 or OKT5/8 (6-8). By this time it had become appreciated that Lyt-1 in the mouse was not reciprocally expressed with Lyt-2; many functional T cells had the Lyt-1⁺2⁺ phenotype (5, 8, 9), and all T cells expressed some Lyt-1 (9-11). In the human, however, a marker termed Leu-3 or OKT4 was found that did show good reciprocal expression with Leu-2 and did not have the molecular characteristics of Lyt-1 (7, 8). An exciting new dimension was added to the study of these molecules with the reports of Shinohara and Sachs (12) and Nakayama *et al.* (13) that antibody to Lyt-2 had the unique ability to block most cytotoxic function. This conclusion was quickly confirmed by a number of other workers with monoclonal antibodies (MoAb) (14-16). Studies on the human T cell subsets confirmed this pattern because MoAb to Leu-2 blocked most allogeneic cytotoxicity (17, 18). Most interestingly, in the human, antibody to Leu-3 was able to block proliferation, a response usually associated with helper function (18). Recently we extended this observation to show that anti-Leu-2 blocks cytotoxic function and anti-Leu-3 blocks IL 2 production when human T cells respond to xenogeneic mouse stimulators (19). These studies and several of the details of the blocking, which include the facts that blocking occurs during early events of cell interactions but not during subsequent steps (20, 21), and that the lectins such as concan-

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² Abbreviations: MHC, major histocompatibility complex; MoAb, monoclonal antibody.

avalin A (Con A) and phytohemagglutinin can overcome blocking (14, 22), fueled speculation that Lyt and Leu molecules were active in T cell recognition.

The original descriptions of these Lyt and Leu molecules tended to suggest that they were markers associated with T cell function. Although there is a strong association between phenotype and function, there is also a correlation between Lyt/Leu phenotype and the class of MHC molecule to which the T cells are directed. We first raised the possibility that Lyt phenotype showed a better correlation with the class of MHC molecule recognized by the T cell than with T cell function in studies involving allogeneic responses to different MHC subregions (9, 16, 23). It was possible in the allogeneic situation to study the Lyt phenotype of allogeneic helpers specific for either class 1 or class 2 MHC antigens and of cytotoxic effectors with similar subregion specificities. In all of these situations, the expression of Lyt-2 was correlated with class 1 MHC recognition rather than with function (16, 23). These studies were later extended to include a T cell line with cytotoxic activity that was specific for class 2 (24). Finally we tested the ability of MoAb to Lyt-2 to block these subregion-specific responses, and again the blocking was correlated with recognition of class 1 molecules rather than with function (16). This set of phenomena was incomplete, however, because no reciprocal molecule associated with class 2 MHC recognition was known and antibodies directed to Lyt-1 did not block T cell responses (14, 16).

Experiments in the human systems designed to study the association of Leu expression as well as the ability of MoAb to Leu molecules to block function again confirmed and extended the phenomena in the murine system. It was found in several independent studies of human T cell lines that Leu-2 expression and the ability of anti-Leu-2 to block was strongly associated with recognition of class 1 HLA molecules, whereas Leu-3 expression and the ability of anti-Leu-3 to block was strongly associated with recognition of class 2 HLA molecules (25-27).

The association of Lyt and Leu expression with MHC subregion recognition and the unusual ability of MoAb to these structures to block T cell function are strong evidence for some involvement of these molecules in T cell-MHC interaction, and further suggest that these molecules might play a role in discriminating class 1 from class 2 MHC molecules.

Recently Dialynas and colleagues (26, 28) developed a MoAb with specificity for murine T cell surface molecules that are reciprocally expressed with Lyt-2 and have the same gross biochemical characteristics in electrophoretic analysis as Leu-3 or OKT4 molecules in the human, with an apparent m.w. of 52,000. We report here that this anti-L3T4 reagent blocks primary and secondary helper T cell responses to class 2 molecules but not to class 1 molecules. These data and data (29, 30) obtained with hybridomas and cell lines provide further evidence that L3T4 is the mouse equivalent of human Leu-3 and OKT4 and gives added weight to the hypothesis that Lyt-2, L3T4, and Leu-2, Leu-3 molecules play an important role in T cell interaction with MHC molecules.

MATERIALS AND METHODS

Mice. All inbred mice were bred in our colony at the University of California, San Diego. Original breeding pairs were obtained from

the Jackson Laboratory, Bar Harbor, ME, and from Dr. Donald Shreffler (Washington University School of Medicine, St. Louis, MO).

Determination of allohelp. Allohelp is operationally defined as the striking ability of mitomycin-treated T cells to interact with allogeneic T-depleted B cells to cause them to become antibody-forming cells in *in vitro* cultures. Mitomycin C-treated T cells synergistic to B cells give virtually no help. The cultures are constructed as reported previously (23). Briefly, T cells are obtained by nylon column passage and are mitomycin C-treated ($25 \mu\text{g}/5 \times 10^7$ T cells). B cells are obtained by treating spleen cells with MoAb to Thy-1.2 (F7D5) plus C. T cells are titrated (5×10^5 to 1×10^4) into 0.1-ml cultures containing 6×10^5 B cells and 0.1% v/v selected sheep erythrocytes (SRBC, Colorado Serums, Denver, CO). Direct (IgM) plaque-forming cells (PFC) to SRBC are determined after 4 days of culture. Geometric means (\pm standard errors) of triplicate cultures are determined. All cells are cultured in RPMI 1640 supplemented with penicillin, streptomycin, and glutamine with 5% selected fetal bovine serum.

In vitro priming. Priming of T cells to MHC subregion differences is accomplished *in vitro* as described (31). Briefly, 2.5×10^7 spleen cells of the responding strain are cultured with an equal number of mitomycin C-treated cells of the stimulating strain in a total volume of 20 ml. After 10 to 14 days, cultures are harvested and restimulated. Such priming typically results in at least a 10- to 20-fold increase in the specific response and loss of third party responses (31).

Determination of IL 2 production. The cultures described above containing primed T cells are cultured at $2.5 \times 10^5/\text{ml}$ with $5 \times 10^6/\text{ml}$ of anti-Thy-1.2 (F7D5) plus C, mitomycin C-treated stimulators, and the supernatants are collected after 16 to 20 hr. The presence of IL 2 is detected by adding the supernatants at concentrations from 50 to 1% to triplicate cultures of 5×10^5 IL 2-dependent T cells from an NK line, as described previously (32). Proliferation of cells was determined by incorporation of ^3H -thymidine deoxyribose added during the last 24 hr of a 48-hr culture (32). Results are expressed as the mean cpm of triplicate cultures.

Blocking with MoAb. Culture supernatants from *in vitro* cultured hybridomas producing antibody to Lyt-2, L3T4, and LFA-1 (details below) were added at the initiation of the cultures of allohelpers and B cells or at the initiation of cultures for the production of IL 2. Concentrations of such antibodies ranged from 20 to 2% for the allohelp cultures and from 5 to 1% for IL 2 production.

MoAb, Anti-Thy-1.2 (F7D5) was a kind gift of Phil Lake. The culture supernatants containing anti-Lyt-2 were obtained from the rat hybridoma 53.6.72, which was obtained from the Salk Institute Cell Culture Facility and was donated by Drs. Ledbetter and Herzenberg. The culture supernatants containing anti-L3T4 were obtained from the GK1.5 hybridoma (30) and the culture supernatants containing anti-LFA-1 were from the FD4418 hybridoma (22) of Dr. Dialynas.

RESULTS

Anti-Lyt-2 and anti-L3T4 reciprocally block allogeneic help specific for class 1 and class 2 MHC antigens, respectively. The effect of anti-Lyt-2 and anti-L3T4 on allogeneic help specific for class 1 or class 2 MHC antigens was determined. T cells were taken from spleens of inbred mice and titrated into cultures of T-depleted spleen cells from allogeneic inbred mice as a source of B cells. Inbred strains were chosen that differed only in H-2K or H-2D (class 1) or at the I region (class 2). Anti-Lyt-2, anti-L3T4, or anti-LFA-1 were included at various concentrations in parallel cultures. In all cases, the allohelp was assessed by the number of PFC per culture that developed in 4 days to SRBC as antigen. The results of a representative example of such an experiment are illustrated in Figure 1. In Figure 1a, B6 T cells were titrated against B cells of the B6.bml strain, which express a mutant form of the K^b molecule. This strain combination gives a very strong allogeneic helper response even though the difference in MHC is restricted to class 1 antigens alone. Culture supernatant containing MoAb to Lyt-2 completely abrogated this response. In contrast, culture supernatants containing MoAb to L3T4 had no significant effect on allogeneic help. Antibody to LFA-1

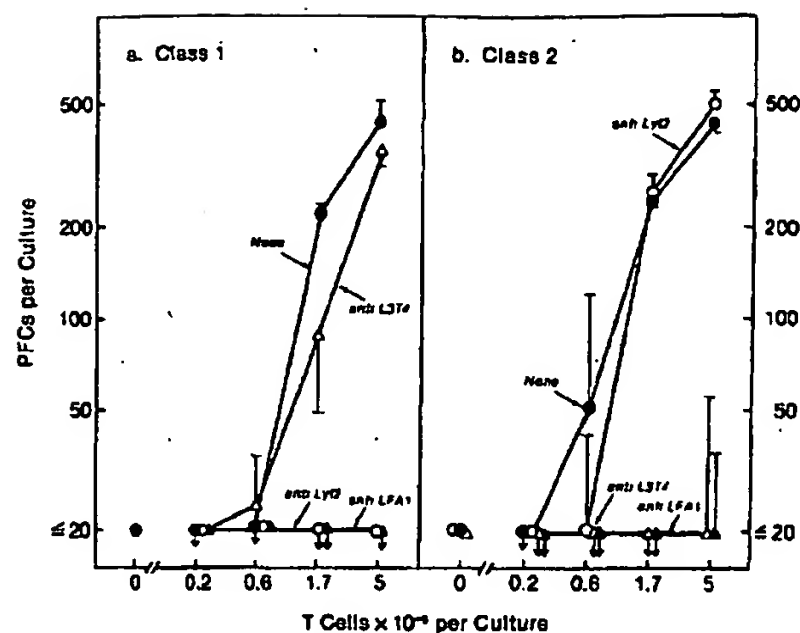


Figure 1. Blocking of MHC specific allohelp. *a*, mitomycin-treated B6 T cells were titrated at numbers indicated into triplicate cultures containing 6×10^6 B cells (T-depleted spleen) from B6.bm1 mice. Cultures were set up with no addition (\bullet); with 2% culture supernatant from the GK1.5 line (anti-L3T4) (Δ); with 2% culture supernatant from the 53.6.72 line (anti-Lyt-2) (Δ); or with 2% culture supernatant from the FD4418 line (anti-LFA-1) (\circ). Mean PFC per culture \pm SEM are indicated. *b*, mitomycin-treated B10.AQR T cells were titrated into triplicate cultures of B10.T(6R) B cells as in *a*. The same additions were made as in *a*.

TABLE I
Reciprocal blocking of allohelp by MoAb to Lyt-2 and L3T4

	T Cells	B Cells	MoAb Specificity	PFC/Culture
Class 1-specific				
Expt. 1	-	B6.bm1	-	2 (1-2)
	B6	+	-	203 (181-227)
	+	+	L3T4	271 (228-323)
	+	+	Lyt-2	24 (20-28)
	+	+	LFA-1	<2
Expt. 2	-	B10.T(6R)	-	<2
	B10.G	+	-	83 (70-99)
	+	+	L3T4	60 (56-64)
	+	+	Lyt-2	9 (4-19)
	+	+	LFA-1	<2
Class 2 specific or whole haplotype				
Expt. 1	-	B10.AQR	-	<2
	B10.T(6R)	+	-	36 (26-48)
	+	+	L3T4	6 (3-9)
	+	+	Lyt-2	30 (25-38)
	+	+	LFA-1	4 (2-11)
Expt. 2	-	BDF ₁	-	1056 (1024-1087)
	B6	+	-	98 (91-106)
	+	+	L3T4	626 (556-704)
	+	+	Lyt-2	3 (2-5)
	+	+	LFA-1	11 (7-17)
Mis-specific				
Expt. 1	-	D1.LP	-	149 (120-184)
	B6	+	-	614 (442-853)
	+	+	L3T4	45 (35-57)
	+	+	Lyt-2	791 (711-880)
	+	+	LFA-1	11 (7-17)

was also very effective in preventing a PFC response. In Figure 1b, B10.T(6R) T cells were titrated against B cells from B10.AQR mice. These strains differ over the entire I region. Again a very strong allogeneic helper response was seen. In this case, however, the same preparations of MoAb had strikingly different effects. MoAb to Lyt-2 had no effect on the response, whereas MoAb to L3T4 completely blocked responsiveness. MoAb to LFA-1 also completely blocked the allogeneic help. Similar results were seen in three other experiments with the same strain combination. Results of some of these experiments are included in Table I.

Several more comments can be made. First, when other recombinant inbred strains of mice were used as T and B cell donors differing only at class 1 MHC, weaker

allogeneic help was seen (see Table I) as reported previously (23). However, the pattern of anti-Lyt-2 and not anti-L3T4 blocking was the same regardless of whether the response was a strong one (reflecting a high frequency of T cells specific for the K^b mutant (33)) or a weaker one (reflecting the generally lower frequency of class 1-specific allohelpers). As expected, the response to a whole MHC region was susceptible to anti-L3T4 and was largely resistant to anti-Lyt-2 (Table I). Although a 40% lower response was seen in the cultures with anti-Lyt-2, this degree of blocking is not reproducible over a large number of experiments (not shown, but note previous results especially Reference 23).

Ability of anti-L3T4 to block class 2 but not class 1-specific allohelp. Table II shows a titration of anti-L3T4 antibody into a response against class 2 MHC (B10.T(6R) T cells with B10.AQR B cells) and against class 1 MHC (B10.G to B10.T(6R)). It is clear that MoAb to L3T4 showed specific blocking of T cells directed to class 2 MHC over a broad range of doses. A slight inhibition of class 1 responses was seen with the addition of L3T4 supernatants, but this sort of inhibition may reflect variation among triplicate cultures (which can be as much as twofold), and did not appear to increase with increasingly larger doses of L3T4, suggesting it is nonspecific.

Anti-Lyt-2 and anti-L3T4 reciprocally block IL 2 production by T cells primed and restimulated in vitro with class 1 and class 2 differences, respectively. T cells were primed in vitro to either class 1 or class 2 MHC subregion differences. Such T cells showed a much en-

TABLE II
Ability of anti-L3T4 to block class 2, but not class 1, specific allohelp

B Cells	T Cells	Addition	PFC/Culture
B10.AQR	B10.T(6R)	None	1236 (170-1884)
		Anti-L3T4, 20%	14 (3-34)
		10%	28 (17-48)
		5%	15 (9-24)
		2%	46 (20-106)
B10.G	B10.T(6R)	None	549 (427-705)
		Anti-L3T4, 20%	355 (315-400)
		10%	331 (291-376)
		5%	185 (160-215)
		2%	303 (285-320)

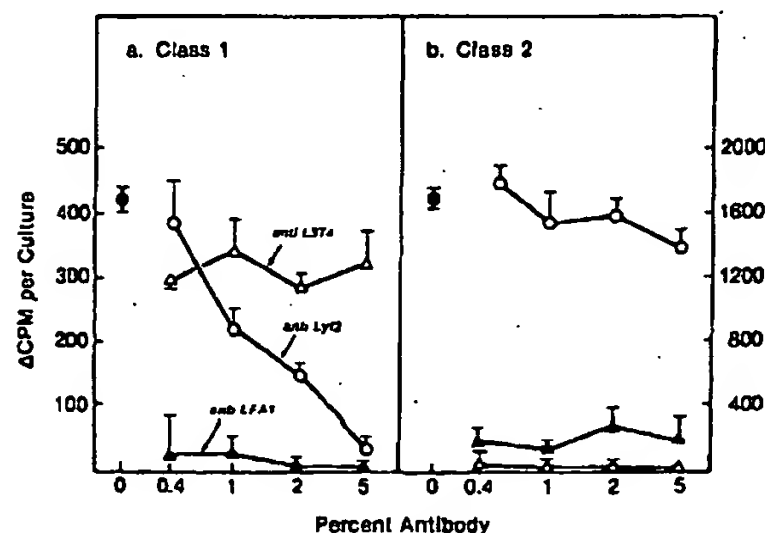


Figure 2. Blocking of secondary class 1 or 2 MHC-specific responses. *a*, in vitro primed B10.T(6R) T cells were restimulated in vitro with B10.G B cells. Cultures were incubated with indicated doses of supernatants from GK1.5 (anti-L3T4) (Δ); 53.6.72 (anti-Lyt-2) (\circ); and FD4418 (anti-LFA-1) (Δ). Culture supernatants were collected after 18 hr and their content of IL 2 was assayed by the ability of 17% of supernatant to cause the proliferation (Δ cpm per culture) of an IL 2-dependent line. *b*, conditions were identical to *a* except that B10.T(6R) T cells were primed and restimulated with B10.AQR B cells.

hanced response to the priming MHC subregion difference and have lost most responsiveness to third party MHC antigens as detected both by their ability to give allohelp and by their production of the lymphokine IL 2 in response to stimulation to the priming alloantigen (31). MoAb to Lyt-2 and L3T4 were included during the restimulation with T-depleted stimulators of the priming haplotype, and supernatants were collected after 18 to 24 hr and their IL 2 content was determined. It was previously shown that the presence of these MoAb did not affect the IL 2 assay (16). The results of a representative experiment are illustrated by Figure 2. The supernatants were all titrated in the IL 2 assay, but only the proliferation of the NK targets at 17% added supernatants is shown. Similar effects were seen at 50 and 6%. Production of IL 2 in response to class 1 MHC antigen (B10.T(6R) against B10.G) in this case was blocked by MoAb to Lyt-2 but not to L3T4. In contrast, MoAb to Lyt-2 had no effect on the IL 2 production of T cells responding to class 2, whereas L3T4 was very effective at blocking in this situation. MoAb to LFA-1 blocked IL 2 production effectively in both situations. This result was found three times with these strain combinations.

DISCUSSION

The results in this report show clearly that MoAb to L3T4 and to Lyt-2 reciprocally block allohelp and lymphokine production of normal bulk cultures of T cells specific for class 2 and class 1 MHC antigens, respectively. Previously, MoAb to L3T4 was reported to interact with a T cell surface molecule that is expressed on normal T cells and in most T cell lines and hybridomas reciprocally with Lyt-2 (28-30). The molecule precipitated by the anti-L3T4 has an apparent m.w. of 52,000 and behaves similarly to Leu-3/OKT4 on polyacrylamide gel electrophoresis (28). In addition, anti-L3T4 was found to block the class 2 MHC-specific cytolytic line A15-1.17 (28, 30, 34). Thus there is good evidence that L3T4 is the murine homologue of Leu-3/OKT4, and furthermore, that the murine and human T cell surface markers might be involved in a similar fashion in interaction with class 2 MHC molecules.

The results in this paper extend this picture to bulk populations of normal T cells. With the previously reported results indicating Lyt-2 blocking of class 1 directed response regardless of T cell function, and that human T cell lines specific for class 1 expressed and were blocked by anti-Leu-2, while lines specific for class 2 expressed and were blocked by anti-Leu-3, a striking pattern emerges that is similar in mouse and man. In both species there are T cell surface molecules of similar structure strongly associated in a class-specific manner with recognition of MHC antigens. In both cases, some MoAb to these structures have the unique property of blocking the interaction of T cells with antigen-presenting cells or target cells at an early stage, which correlates temporally with recognition events.

Furthermore, as we reported previously, the xenogeneic interactions that occur when human T cells recognize mouse MHC molecules are appropriately blocked by MoAb to Leu-2 and Leu-3 structures on such human T cells (19).

In the studies in this report, the MoAb to LFA-1 consistently blocked all T cell responses, regardless of spec-

ificity. This pattern of blocking is consistent with those reported for anti-LFA-1 by Springer and colleagues (35) and other investigators (36) (D. W. Lancki et al., manuscript in preparation) and in marked contrast to the reciprocal blocking of anti-Lyt-2 and anti-L3T4, which depended on whether T cells were responding to class 1 or class 2. It is important to note that antibodies to LFA-1 and the Lyt and Leu families of molecules are the only antibodies to T cell surface markers that have been found consistently to block function except for the recent reports of clone-specific MoAb, which appear to be directed to the antigen-specific portion(s) of the cell receptor (37, 38), and antibodies to the human Leu-4/OKT3 molecule, which appear to be associated with the clonotypic receptor (41).

The impressive nature of the blocking we report in bulk cultures of T cells indicates that the vast majority of T cells taking part in the allogeneic help and lymphokine production *in situ* are susceptible to the blocking effects of the Lyt/Leu-specific MoAb, and imply that these structures or something physically associated with them are required for the interaction of the T cell with MHC molecules.

In the case of primed T cells and T cell clones, a number of investigators have described instances in which the appropriate MoAb has failed to block function, even though T cells of appropriate specificity were involved (21, 39). These observations put important constraints on the interpretation of the findings outlined above. They suggest that the interaction of Lyt/Leu molecules with MHC class-specific determinants is not always obligatory. In many cases these failures of blocking could be ascribed to interactions between T cell receptors and antigen, being of sufficient affinity to overcome blocking, as suggested by MacDonald and colleagues (21). Certainly there is considerable heterogeneity among T cell clones with respect to their susceptibility to blocking with anti-Lyt-2. MacDonald et al., however, have also presented evidence that certain T cell clones specific for class 1 molecules have no detectable expression of Lyt-2 (21). Because of the virtually complete suppression of normal bulk responses seen here and in our previous studies (16), it seems that these T cells must be rare in unprimed populations. We think the most feasible way to reconcile these findings is to postulate that Lyt-2 and L3T4 molecules in the mouse and Leu-2 and Leu-3 molecules in the human do interact with MHC molecules of class 1 and class 2, but that this interaction is not directly required for T cell triggering; instead it increases the relative avidity of the binding of the antigen-specific T cell receptor with specific antigen, including polymorphic "restricting" determinants on MHC. An analysis of spontaneously arising L3T4 loss variants of cloned class 2 MHC-reactive T cell hybridomas is consistent with the model (P. Marrack et al., manuscript in preparation). Similar concepts have been proposed in other reports by us (19, 40) and by MacDonald et al. (21), Reinherz et al. (41), and Biddison et al. (27).

The alternative explanation, made increasingly unlikely with the large amount of recent data showing a similar pattern of association of Lyt and Leu with recognition of MHC class in diverse systems, is that these T cell surface markers are not involved in interaction themselves but are "spatially" associated with structures re-

quired in T cell function, or somehow give negative signals to the cell. Although such possibilities should not be dismissed, we believe that the specificity of these molecules for either class 1 or class 2 directed interactions, their impressive evolutionary conservation revealed by the striking similarities of the murine and human systems, and the relative uniqueness of cell surface markers against which blocking antibodies can be raised, as well as the mechanistic data that indicate blocking occurs at the level of T cell interaction with other cells, provide a large amount of circumstantial evidence that the Lyt and Leu molecules are directly involved in interaction with MHC antigens.

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MONOCLONAL ANTIBODY TO L3T4 BLOCKS THE FUNCTION OF T CELLS SPECIFIC FOR CLASS 2 MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS¹

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The ability of antibody to Lyt-2 and to the newly described L3T4 antigen to block the functions of helper T cells with specificity for allogeneic class 1 or class 2 MHC subregion antigens was determined. Anti-Lyt-2 blocked both allohelp delivered by unprimed T cells and IL 2 production by primed T cells when the response was directed to class 1 MHC antigens, but had no effect when the response was directed to class 2 MHC or Mls antigens. Anti-L3T4 had reciprocal activity. A control reagent, anti-LFA-1, blocked all responses tested regardless of specificity. This and other reports provide strong evidence that L3T4 is the murine equivalent of the human T cell markers Leu 3/OKT4. The ability of antibodies directed against Lyt-2 and L3T4 to block T cell function in a fashion determined by the class of MHC antigen recognized by the T cell further supports the hypotheses that Lyt-2 and L3T4 molecule in the mouse and Leu-2 and Leu-3 molecules in the human are involved in the interaction of the T cell with class-specific determinants on MHC molecules.

It has become clear in the past decade that most, if not all, helper and cytotoxic T cells must recognize and interact with major histocompatibility complex (MHC)² molecules to function. This requirement is reflected in non-MHC antigen-specific responses by the phenomenon of MHC restriction and in nonhomologous responses in the very large T cell response to allogeneic and xenogeneic MHC antigens.

There are several striking features both of the T cell response to MHC antigens and of the MHC antigens themselves. First there are two classes of MHC molecules, which are found in all species studied and are of quite different structure; these are termed class 1 and class 2 antigens (reviewed in Reference 1). The class 1 molecules, the original transplantation antigens, consist of a single transmembrane MHC-encoded polymorphic chain of 40,000 to 45,000 m.w. associated with non-MHC-encoded β_2 -microglobulin chain. In the mouse, the class

1 molecules include the well-studied K and D-encoded molecules. The recognition of these class 1 molecules is generally correlated with T cells that have cytotoxic function. The class 2 molecules consist of two MHC-encoded transmembrane chains of approximately 34,000 and 28,000 m.w., termed α and β -chains. These correspond to the I region molecules of the mouse, which include the I-A and I-E region molecules. The recognition of class 2 molecules is generally correlated with T cells that have helper or inducer function (1-4). Thus there is a functional as well as chemical distinction between class 1 and class 2 MHC molecules in their role as antigens recognized by T cells.

Subsets of T cells have also been identified by their expression of a series of surface markers termed Lyt in the mouse and Leu or OKT in the human. It was appreciated quite early that expression of these markers on T cells showed a good association with function. Thus, in the mouse, the subset of T cells expressing Lyt-1, but not expressing Lyt-2, was found to contain the major fraction of helper activity, whereas that subset expressing Lyt-2 contained most T cells capable of cytotoxic function (5). This pattern was later reproduced in studies on human T cells, which also yielded new and important insights. Human marker(s) were found that corresponded to Lyt-2 called Leu 2 or OKT5/8 (6-8). By this time it had become appreciated that Lyt-1 in the mouse was not reciprocally expressed with Lyt-2; many functional T cells had the Lyt-1⁺2⁺ phenotype (5, 8, 9), and all T cells expressed some Lyt-1 (9-11). In the human, however, a marker termed Leu-3 or OKT4 was found that did show good reciprocal expression with Leu-2 and did not have the molecular characteristics of Lyt-1 (7, 8). An exciting new dimension was added to the study of these molecules with the reports of Shinohara and Sachs (12) and Nakayama *et al.* (13) that antibody to Lyt-2 had the unique ability to block most cytotoxic function. This conclusion was quickly confirmed by a number of other workers with monoclonal antibodies (MoAb) (14-16). Studies on the human T cell subsets confirmed this pattern because MoAb to Leu-2 blocked most allogeneic cytotoxicity (17, 18). Most interestingly, in the human, antibody to Leu-3 was able to block proliferation, a response usually associated with helper function (18). Recently we extended this observation to show that anti-Leu-2 blocks cytotoxic function and anti-Leu-3 blocks IL 2 production when human T cells respond to xenogeneic mouse stimulators (19). These studies and several of the details of the blocking, which include the facts that blocking occurs during early events of cell interactions but not during subsequent steps (20, 21), and that the lectins such as concan-

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² Abbreviations: MHC, major histocompatibility complex; MoAb, monoclonal antibody.

avalin A (Con A) and phytohemagglutinin can overcome blocking (14, 22), fueled speculation that Lyt and Leu molecules were active in T cell recognition.

The original descriptions of these Lyt and Leu molecules tended to suggest that they were markers associated with T cell function. Although there is a strong association between phenotype and function, there is also a correlation between Lyt/Leu phenotype and the class of MHC molecule to which the T cells are directed. We first raised the possibility that Lyt phenotype showed a better correlation with the class of MHC molecule recognized by the T cell than with T cell function in studies involving allogeneic responses to different MHC subregions (9, 16, 23). It was possible in the allogeneic situation to study the Lyt phenotype of allogeneic helpers specific for either class 1 or class 2 MHC antigens and of cytotoxic effectors with similar subregion specificities. In all of these situations, the expression of Lyt-2 was correlated with class 1 MHC recognition rather than with function (16, 23). These studies were later extended to include a T cell line with cytotoxic activity that was specific for class 2 (24). Finally we tested the ability of MoAb to Lyt-2 to block these subregion-specific responses, and again the blocking was correlated with recognition of class 1 molecules rather than with function (16). This set of phenomena was incomplete, however, because no reciprocal molecule associated with class 2 MHC recognition was known and antibodies directed to Lyt-1 did not block T cell responses (14, 16).

Experiments in the human systems designed to study the association of Leu expression as well as the ability of MoAb to Leu molecules to block function again confirmed and extended the phenomena in the murine system. It was found in several independent studies of human T cell lines that Leu-2 expression and the ability of anti-Leu-2 to block was strongly associated with recognition of class 1 HLA molecules, whereas Leu-3 expression and the ability of anti-Leu-3 to block was strongly associated with recognition of class 2 HLA molecules (25-27).

The association of Lyt and Leu expression with MHC subregion recognition and the unusual ability of MoAb to these structures to block T cell function are strong evidence for some involvement of these molecules in T cell-MHC interaction, and further suggest that these molecules might play a role in discriminating class 1 from class 2 MHC molecules.

Recently Dialynas and colleagues (26, 28) developed a MoAb with specificity for murine T cell surface molecules that are reciprocally expressed with Lyt-2 and have the same gross biochemical characteristics in electrophoretic analysis as Leu-3 or OKT4 molecules in the human, with an apparent m.w. of 52,000. We report here that this anti-L3T4 reagent blocks primary and secondary helper T cell responses to class 2 molecules but not to class 1 molecules. These data and data (29, 30) obtained with hybridomas and cell lines provide further evidence that L3T4 is the mouse equivalent of human Leu-3 and OKT4 and gives added weight to the hypothesis that Lyt-2, L3T4, and Leu-2, Leu-3 molecules play an important role in T cell interaction with MHC molecules.

MATERIALS AND METHODS

Mice. All inbred mice were bred in our colony at the University of California, San Diego. Original breeding pairs were obtained from

the Jackson Laboratory, Bar Harbor, ME, and from Dr. Donald Shreffler (Washington University School of Medicine, St. Louis, MO).

Determination of allohelp. Allohelp is operationally defined as the striking ability of mitomycin-treated T cells to interact with allogeneic T-depleted B cells to cause them to become antibody-forming cells in *in vitro* cultures. Mitomycin C-treated T cells syngeneic to B cells give virtually no help. The cultures are constructed as reported previously (23). Briefly, T cells are obtained by nylon column passage and are mitomycin C-treated ($25 \mu\text{g}/5 \times 10^7$ T cells). B cells are obtained by treating spleen cells with MoAb to Thy-1.2 (F7D5) plus C. T cells are titrated (5×10^5 to 1×10^4) into 0.1-ml cultures containing 6×10^5 B cells and 0.1% v/v selected sheep erythrocytes (SRBC, Colorado Serums, Denver, CO). Direct (IgM) plaque-forming cells (PFC) to SRBC are determined after 4 days of culture. Geometric means (\pm standard errors) of triplicate cultures are determined. All cells are cultured in RPMI 1640 supplemented with penicillin, streptomycin, and glutamine with 5% selected fetal bovine serum.

In vitro priming. Priming of T cells to MHC subregion differences is accomplished *in vitro* as described (31). Briefly, 2.5×10^7 spleen cells of the responding strain are cultured with an equal number of mitomycin C-treated cells of the stimulating strain in a total volume of 20 ml. After 10 to 14 days, cultures are harvested and restimulated. Such priming typically results in at least a 10- to 20-fold increase in the specific response and loss of third party responses (31).

Determination of IL 2 production. The cultures described above containing primed T cells are cultured at $2.5 \times 10^5/\text{ml}$ with $5 \times 10^5/\text{ml}$ of anti-Thy-1.2 (F7D5) plus C, mitomycin C-treated stimulators, and the supernatants are collected after 16 to 20 hr. The presence of IL 2 is detected by adding the supernatants at concentrations from 50 to 1% to triplicate cultures of 5×10^5 IL 2-dependent T cells from an NK line, as described previously (32). Proliferation of cells was determined by incorporation of ^3H -thymidine deoxyribose added during the last 24 hr of a 48-hr culture (32). Results are expressed as the mean cpm of triplicate cultures.

Blocking with MoAb. Culture supernatants from *in vitro* cultured hybridomas producing antibody to Lyt-2, L3T4, and LFA-1 (details below) were added at the initiation of the cultures of allohelpers and B cells or at the initiation of cultures for the production of IL 2. Concentrations of such antibodies ranged from 20 to 2% for the allohelp cultures and from 5 to 1% for IL 2 production.

MoAb. Anti-Thy-1.2 (F7D5) was a kind gift of Phil Lake. The culture supernatants containing anti-Lyt-2 were obtained from the rat hybridoma 53.6.72, which was obtained from the Salk Institute Cell Culture Facility and was donated by Drs. Ledbetter and Herzenberg. The culture supernatants containing anti-L3T4 were obtained from the GK1.5 hybridoma (30) and the culture supernatants containing anti-LFA-1 were from the FD4418 hybridoma (22) of Dr. Dialynas.

RESULTS

Anti-Lyt-2 and anti-L3T4 reciprocally block allogeneic help specific for class 1 and class 2 MHC antigens, respectively. The effect of anti-Lyt-2 and anti-L3T4 on allogeneic help specific for class 1 or class 2 MHC antigens was determined. T cells were taken from spleens of inbred mice and titrated into cultures of T-depleted spleen cells from allogeneic inbred mice as a source of B cells. Inbred strains were chosen that differed only in H-2K or H-2D (class 1) or at the I region (class 2). Anti-Lyt-2, anti-L3T4, or anti-LFA-1 were included at various concentrations in parallel cultures. In all cases, the allohelp was assessed by the number of PFC per culture that developed in 4 days to SRBC as antigen. The results of a representative example of such an experiment are illustrated in Figure 1. In Figure 1a, B6 T cells were titrated against B cells of the B6.bm1 strain, which express a mutant form of the K^b molecule. This strain combination gives a very strong allogeneic helper response even though the difference in MHC is restricted to class 1 antigens alone. Culture supernatant containing MoAb to Lyt-2 completely abrogated this response. In contrast, culture supernatants containing MoAb to L3T4 had no significant effect on allogeneic help. Antibody to LFA-1

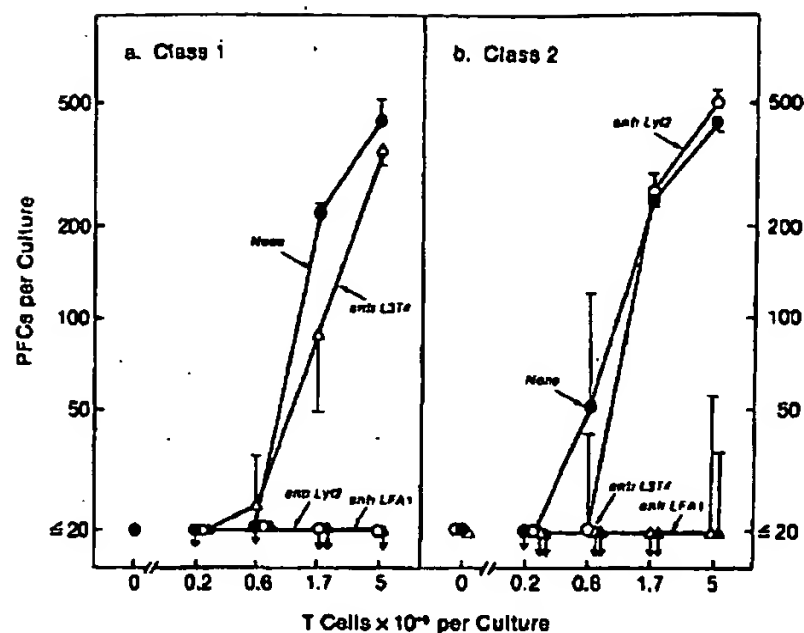


Figure 1. Blocking of MHC specific allohelp. *a*, mitomycin-treated B6 T cells were titrated at numbers indicated into triplicate cultures containing 6×10^6 B cells (T-depleted spleen) from B6.bm1 mice. Cultures were set up with no addition (\bullet); with 2% culture supernatant from the GK1.5 line (anti-L3T4) (Δ); with 2% culture supernatant from the 53.6.72 line (anti-Lyt-2) (\square); or with 2% culture supernatant from the FD4418 line (anti-LFA-1) (\circ). Mean PFC per culture \pm SEM are indicated. *b*, mitomycin-treated B10.AQR T cells were titrated into triplicate cultures of B10.T(6R) B cells as in *a*. The same additions were made as in *a*.

TABLE I
Reciprocal blocking of allohelp by MoAb to Lyt-2 and L3T4

	T Cells	B Cells	MoAb Specificity	PFC/Culture
Class 1-specific				
Expt. 1	-	B6.bm1	-	2 (1-2)
	B6	+	-	203 (181-227)
	+	+	L3T4	271 (228-323)
	+	+	Lyt-2	24 (20-28)
Expt. 2	-	B10.T(6R)	-	<2
	B10.G	+	-	83 (70-99)
	+	+	L3T4	60 (56-64)
	+	+	Lyt-2	9 (4-19)
Class 2 specific or whole haplotype				
Expt. 1	-	B10.AQR	-	<2
	B10.T(6R)	+	-	36 (26-48)
	+	+	L3T4	6 (3-9)
	+	+	Lyt-2	30 (25-38)
Expt. 2	-	BDF ₁	-	4 (2-11)
	B6	+	-	1056 (1024-1087)
	+	+	L3T4	98 (91-106)
	+	+	Lyt-2	626 (556-704)
Mis-specific	-	D1.LP	-	3 (2-5)
	B6	+	-	149 (120-184)
	+	+	L3T4	614 (442-853)
	+	+	Lyt-2	45 (35-57)
Expt. 1	-	D1.LP	-	791 (711-880)
	B6	+	-	11 (7-17)
	+	+	L3T4	791 (711-880)
	+	+	LFA-1	11 (7-17)

was also very effective in preventing a PFC response. In Figure 1*b*, B10.T(6R) T cells were titrated against B cells from B10.AQR mice. These strains differ over the entire I region. Again a very strong allogeneic helper response was seen. In this case, however, the same preparations of MoAb had strikingly different effects. MoAb to Lyt-2 had no effect on the response, whereas MoAb to L3T4 completely blocked responsiveness. MoAb to LFA-1 also completely blocked the allogeneic help. Similar results were seen in three other experiments with the same strain combination. Results of some of these experiments are included in Table I.

Several more comments can be made. First, when other recombinant inbred strains of mice were used as T and B cell donors differing only at class 1 MHC, weaker

allogeneic help was seen (see Table I) as reported previously (23). However, the pattern of anti-Lyt-2 and not anti-L3T4 blocking was the same regardless of whether the response was a strong one (reflecting a high frequency of T cells specific for the K^b mutant (33)) or a weaker one (reflecting the generally lower frequency of class 1-specific allohelpers). As expected, the response to a whole MHC region was susceptible to anti-L3T4 and was largely resistant to anti-Lyt-2 (Table I). Although a 40% lower response was seen in the cultures with anti-Lyt-2, this degree of blocking is not reproducible over a large number of experiments (not shown, but note previous results especially Reference 23).

Ability of anti-L3T4 to block class 2 but not class 1-specific allohelp. Table II shows a titration of anti-L3T4 antibody into a response against class 2 MHC (B10.T(6R) T cells with B10.AQR B cells) and against class 1 MHC (B10.G to B10.T(6R)). It is clear that MoAb to L3T4 showed specific blocking of T cells directed to class 2 MHC over a broad range of doses. A slight inhibition of class 1 responses was seen with the addition of L3T4 supernatants, but this sort of inhibition may reflect variation among triplicate cultures (which can be as much as twofold), and did not appear to increase with increasingly larger doses of L3T4, suggesting it is nonspecific.

Anti-Lyt-2 and anti-L3T4 reciprocally block IL 2 production by T cells primed and restimulated *in vitro* with class 1 and class 2 differences, respectively. T cells were primed *in vitro* to either class 1 or class 2 MHC subregion differences. Such T cells showed a much en-

TABLE II
Ability of anti-L3T4 to block class 2, but not class 1, specific allohelp

B Cells	T Cells	Addition	PFC/Culture
B10.AQR	B10.T(6R)	None	1236 (170-1884)
		Anti-L3T4, 20%	14 (3-34)
		10%	28 (17-48)
		5%	15 (9-24)
		2%	46 (20-106)
B10.G	B10.T(6R)	None	549 (427-705)
		Anti-L3T4, 20%	355 (315-400)
		10%	331 (291-376)
		5%	185 (160-215)
		2%	303 (285-320)

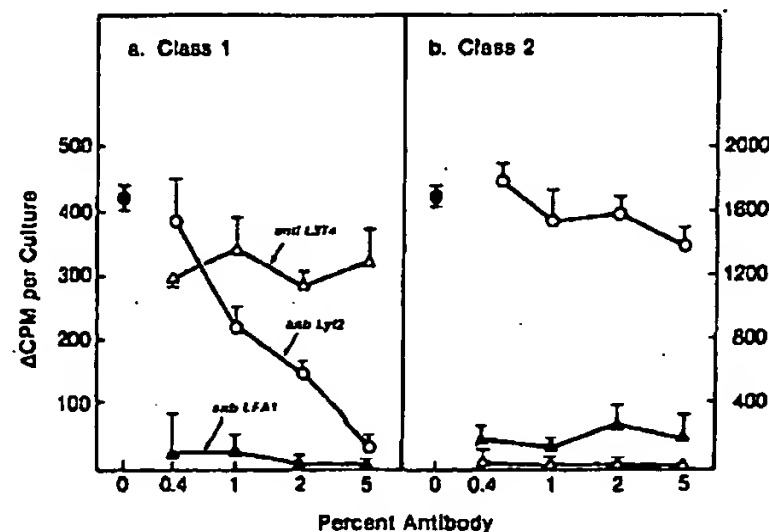


Figure 2. Blocking of secondary class 1 or 2 MHC-specific responses. *a*, *in vitro* primed B10.T(6R) T cells were restimulated *in vitro* with B10.G B cells. Cultures were incubated with indicated doses of supernatants from GK1.5 (anti-L3T4) (Δ); 53.6.72 (anti-Lyt-2) (\circ); and FD4418 (anti-LFA-1) (\square). Culture supernatants were collected after 18 hr and their content of IL 2 was assayed by the ability of 17% of supernatant to cause the proliferation (Δ cpm per culture) of an IL 2-dependent line. *b*, conditions were identical to *a* except that B10.T(6R) T cells were primed and restimulated with B10.AQR B cells.

hanced response to the priming MHC subregion difference and have lost most responsiveness to third party MHC antigens as detected both by their ability to give allohelp and by their production of the lymphokine IL 2 in response to stimulation to the priming alloantigen (31). MoAb to Lyt-2 and L3T4 were included during the restimulation with T-depleted stimulators of the priming haplotype, and supernatants were collected after 18 to 24 hr and their IL 2 content was determined. It was previously shown that the presence of these MoAb did not affect the IL 2 assay (16). The results of a representative experiment are illustrated by Figure 2. The supernatants were all titrated in the IL 2 assay, but only the proliferation of the NK targets at 17% added supernatants is shown. Similar effects were seen at 50 and 6%. Production of IL 2 in response to class 1 MHC antigen (B10.T(6R) against B10.G) in this case was blocked by MoAb to Lyt-2 but not to L3T4. In contrast, MoAb to Lyt-2 had no effect on the IL 2 production of T cells responding to class 2, whereas L3T4 was very effective at blocking in this situation. MoAb to LFA-1 blocked IL 2 production effectively in both situations. This result was found three times with these strain combinations.

DISCUSSION

The results in this report show clearly that MoAb to L3T4 and to Lyt-2 reciprocally block allohelp and lymphokine production of normal bulk cultures of T cells specific for class 2 and class 1 MHC antigens, respectively. Previously, MoAb to L3T4 was reported to interact with a T cell surface molecule that is expressed on normal T cells and in most T cell lines and hybridomas reciprocally with Lyt-2 (28-30). The molecule precipitated by the anti-L3T4 has an apparent m.w. of 52,000 and behaves similarly to Leu-3/OKT4 on polyacrylamide gel electrophoresis (28). In addition, anti-L3T4 was found to block the class 2 MHC-specific cytolytic line A15-1.17 (28, 30, 34). Thus there is good evidence that L3T4 is the murine homologue of Leu-3/OKT4, and furthermore, that the murine and human T cell surface markers might be involved in a similar fashion in interaction with class 2 MHC molecules.

The results in this paper extend this picture to bulk populations of normal T cells. With the previously reported results indicating Lyt-2 blocking of class 1 directed response regardless of T cell function, and that human T cell lines specific for class 1 expressed and were blocked by anti-Leu-2, while lines specific for class 2 expressed and were blocked by anti-Leu-3, a striking pattern emerges that is similar in mouse and man. In both species there are T cell surface molecules of similar structure strongly associated in a class-specific manner with recognition of MHC antigens. In both cases, some MoAb to these structures have the unique property of blocking the interaction of T cells with antigen-presenting cells or target cells at an early stage, which correlates temporally with recognition events.

Furthermore, as we reported previously, the xenogeneic interactions that occur when human T cells recognize mouse MHC molecules are appropriately blocked by MoAb to Leu-2 and Leu-3 structures on such human T cells (19).

In the studies in this report, the MoAb to LFA-1 consistently blocked all T cell responses, regardless of spec-

ificity. This pattern of blocking is consistent with those reported for anti-LFA-1 by Springer and colleagues (35) and other investigators (36) (D. W. Lancki *et al.*, manuscript in preparation) and in marked contrast to the reciprocal blocking of anti-Lyt-2 and anti-L3T4, which depended on whether T cells were responding to class 1 or class 2. It is important to note that antibodies to LFA-1 and the Lyt and Leu families of molecules are the only antibodies to T cell surface markers that have been found consistently to block function except for the recent reports of clone-specific MoAb, which appear to be directed to the antigen-specific portion(s) of the cell receptor (37, 38), and antibodies to the human Leu-4/OKT3 molecule, which appear to be associated with the clonotypic receptor (41).

The impressive nature of the blocking we report in bulk cultures of T cells indicates that the vast majority of T cells taking part in the allogeneic help and lymphokine production *in situ* are susceptible to the blocking effects of the Lyt/Leu-specific MoAb, and imply that these structures or something physically associated with them are required for the interaction of the T cell with MHC molecules.

In the case of primed T cells and T cell clones, a number of investigators have described instances in which the appropriate MoAb has failed to block function, even though T cells of appropriate specificity were involved (21, 39). These observations put important constraints on the interpretation of the findings outlined above. They suggest that the interaction of Lyt/Leu molecules with MHC class-specific determinants is not always obligatory. In many cases these failures of blocking could be ascribed to interactions between T cell receptors and antigen, being of sufficient affinity to overcome blocking, as suggested by MacDonald and colleagues (21). Certainly there is considerable heterogeneity among T cell clones with respect to their susceptibility to blocking with anti-Lyt-2. MacDonald *et al.*, however, have also presented evidence that certain T cell clones specific for class 1 molecules have no detectable expression of Lyt-2 (21). Because of the virtually complete suppression of normal bulk responses seen here and in our previous studies (16), it seems that these T cells must be rare in unprimed populations. We think the most feasible way to reconcile these findings is to postulate that Lyt-2 and L3T4 molecules in the mouse and Leu-2 and Leu-3 molecules in the human do interact with MHC molecules of class 1 and class 2, but that this interaction is not directly required for T cell triggering; instead it increases the relative avidity of the binding of the antigen-specific T cell receptor with specific antigen, including polymorphic "restricting" determinants on MHC. An analysis of spontaneously arising L3T4 loss variants of cloned class 2 MHC-reactive T cell hybridomas is consistent with the model (P. Marrack *et al.*, manuscript in preparation). Similar concepts have been proposed in other reports by us (19, 40) and by MacDonald *et al.* (21), Reinherz *et al.* (41), and Biddison *et al.* (27).

The alternative explanation, made increasingly unlikely with the large amount of recent data showing a similar pattern of association of Lyt and Leu with recognition of MHC class in diverse systems, is that these T cell surface markers are not involved in interaction themselves but are "spatially" associated with structures re-

quired in T cell function, or somehow give negative signals to the cell. Although such possibilities should not be dismissed, we believe that the specificity of these molecules for either class 1 or class 2 directed interactions, their impressive evolutionary conservation revealed by the striking similarities of the murine and human systems, and the relative uniqueness of cell surface markers against which blocking antibodies can be raised, as well as the mechanistic data that indicate blocking occurs at the level of T cell interaction with other cells, provide a large amount of circumstantial evidence that the Lyt and Leu molecules are directly involved in interaction with MHC antigens.

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